THE COLLOIDAL PROPERTIES OF FLUOROCARBON EMULSIONS

by

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ABSTRACT

Some of the colloidal aspects of perfluorochemical emulsions have been investigated. Particular attention was given to the influence of the nature of the oil phase on emulsion stability.

Bulk emulsion stability was measured by an electron micrographic technique. Interfacial and single droplet rest-time data were also collected. A range of surfactants and perfluorochemicals were investigated. It was found that emulsion stability depends on the chemical nature of the oil phase and the emulsifier. The differences in stability could be rationalized in terms of the intermolecular forces between oil molecules, and oil and surfactant molecules.

The effect on stability of a small amount of an additive incorporated into the oil phase was also investigated. It is postulated that although coalescence is the main mechanism by which flourocarbon emulsions coarsen, molecular diffusion (Ostwald Ripening), in the more stable systems, is also important.

Most stable emulsions were obtained by utilizing an emulsifier system comprising a small and a large molecular weight emulsifier.

Accelerated stability testing and optimum storage conditions were also investigated. About $4^{o}C$ was found to be the optimum storage temperature.

The problem of the fluoride ion production during emulsification

could be minimised by sonicating in a carbondioxide atmosphere.

The oxygen uptake and release by fluorocarbon emulsions was rapid, reaching equilibrium within half a second. The in-vitro phagocytosis experiments showed that the phagocytosis rate of fluorocarbon emulsions was dependent on the droplet diameter and its surface characteristics.

Investigation of methods to sterilize the fluorocarbon emulsions showed that filtration of constituents before emulsification coupled with autoclaving had the minimum effect on stability.

A qualitative correlation between single droplet stability and bulk emulsion stability was found and it is concluded that the method could be a useful screening procedure to find an optimum system.

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TO BALBINDER

I. INTRODUCTION

1.1 Pharmaceutical Emulsions

P. Becher (1965) has defined an emulsion as follows:

"An emulsion is a heterogeneous system, consisting of at least one immiscible liquid intimately dispersed in another in the form of droplets whose diameters, in general, exceed 0.1 µm. Such systems possess minimal stability, which may be accentuated by such additives as surface active agents, finely divided solids, etc."

1.1.1 The Emulsion as a dosage form

Emulsions have been used for many years for oral and topical administration of oily substances. For example, fish oils have been rendered more palatable by emulsification since the early nineteenth century. However, widespread use of emulsions, on industrial scale, as drug delivery systems has not been realized because it has been almost impossible to quantify emulsion-stability and drug release characteristics. Nevertheless, many workers have reported the use of oil-in-water (o/w) emulsions to increase the bioavailability of drugs.

Feinstone, Wolff and Williams in 1940 and Daeschner et al in 1957 have reported a more rapid absorption of sulphonamides when administered in an emulsion than in an aqueous suspension. Similarly, Wagner, Gerrard & Kaiser (1966) have reported increased blood levels of an oil soluble substance (indoxole) from an o/w emulsion compared with aqueous suspension or a capsule. Lewis et al (1950) got

enhanced absorption of vitamin A from emulsion. More recently, Carrigan and Bates (1973) found increased bioavailability of griseofulvin from an emulsion. It was found to be oil specific, mineral oils did not give enhanced bioavailability. The physiological basis of this effect was attributed to the modified gastric emptying rate.

In 1974 Kreiglstein, Meffert and Niemeyer reported that, in rabbits, the previous infusion of a fat emulsion into the blood stream protected the animal from high doses of Chlorpromazine. They concluded that the emulsion could take up the lipophylic drugs from the blood stream, thus reducing their concentration and consequently, their availability to the active site. Whether such emulsions could be used clinically in poisoning cases remains to be seen.

Most emulsions when administered orally undergo rapid breakdown on reaching the low pH and high ionic strength of the stomach environment because most emulsifying agents are unstable under these conditions. A detailed study of the bioavailability of drugs given in an emulsion, using different oils and surface active agents and polymers as emulsifiers, should yield some useful data. An alternative approach would be to utilize emulsifying agents which give enhanced stability under conditions of high ionic strength, such as the phosphine oxides and sulphoxides. These are non-ionic agents which absorb ions to give a surface charge, the greater the ionic strength the greater the absorption and therefore greater surface charge giving rise

to enhanced stability. Provided that nontoxic derivatives of these compounds could be found, they could be utilized for intravenous emulsions where, again, the high ionic strength of the plasma can cause instability.

Radiopaque agents have been emulsified and used as diagnostic agents. Kunz, Lewis and Sperandia (1965) have reported preparation, sterilization and stability of oil-in-water emulsions of iodized oil (iophendylate injection) and ethiodized oil (ethiodol). Emulsions of these oils injected intraperitoneally, gave an excellent radiopaque outline of the peritoneal cavity. Iophendylate 50% emulsions injected intrathecally into dogs and into the carpal joint of horse were found to be miscible with the cerebrospinal fluid and synovial fluid respectively, and gave satisfactory radiopacity. More recently, Arambulo et al (1974-1975) have reported the use of highly concentrated o/w emulsions of brominated perfluorocarbon chemicals as radiopaque media. They found these to be non-irritating and useful in bronchography in humans and animals, and angiography in animal studies.

Emulsions have also been used as research tools to study factors affecting phagocytosis. For example, Stossel et al (1972) and Davies et al (1975) have shown that an emulsion can be used as a substrate to measure the rate of phagocytosis by leucocytes. The rate of uptake was shown to be dependent on such factors as the surface charge of emulsion droplets and their size.

Engel and Fahrenbach (1968) and Engel and Riggi (1969) have

reported that o/w emulsions can be used for oral administration of heparin and multiple emulsions (oil-in-water-in-oil) for oral administration of insulin.

1.1.2 Multiple Emulsions

A multiple emulsion is one which contains the primary emulsion, usually water-in-oil, redispersed in an external phase, usually aqueous, to give for example a water-in-oil-in-water emulsion (w/o/w). A multiple emulsion has the advantage of lower viscosity, and therefore is easier to inject, compared with the conventional w/o emulsions which were successfully employed to administer vaccines intramuscularly. The idea of having multiple emulsions was first reported by Herbert (1965) who used it to inject water soluble antigenic material and obtained improved antibody response compared with the original water-in-oil emulsion. However, the final multiple system had to be prepared immediately before injection because of instability. The concept of multiple emulsion has been applied to deliver a variety of chemotherapeutic agents and a sustained release has been reported. Recently Takahashi et al (1976) have reported the administration of an anticancer drug, bleomycin, locally in the form of a multiple emulsion. They found significantly higher concentrations of the drug after intratumoral injection. The anticancer effect was superior than if bleomycin was injected in solution. Clinical trials for treating adenocarcinoma of breast gave favourable results by this technique.

1.1.3 Emulsions for Intravenous Infusion

The use of emulsions infused intravenously can be divided into

two categories. First, for parenteral nutrition and drug delivery, the second, of more recent interest, the use of fluorocarbon emulsions as "artificial blood" substitutes.

1.1.3.1 Parenteral Nutrition and Drug Delivery

Sterile oil-in-water emulsions of purified natural vegetable oils such as soybean oil and, to a lesser extent, cottonseed oil, stabilised by lecithin, are widely used for intravenous nutrition, in Europe. Such emulsions have the advantage of providing large numbers of calories for a small volume and having negligible osmotic effect. The particle size of these emulsions is very important and must not exceed one micron in order to minimise the risk of embolism. This presents problems of stability and preparation of these products. Furthermore, the surfactants and oils must have sufficiently low toxicity, and should not give rise to toxic degradation products during preparation e.g. during sterilisation. For these reasons, the most used substances have been soybean oil and egg-yolk lecithin. However, recently (Jeppsson, 1976) synthetic polyoxyethylene - polyoxypropylene polymers, called "Pluronics" (Pluronic F-68 and F-108) have been utilized to stabilize emulsions which were administered to animals and humans clinically. Davis (1974) has reviewed the pharmaceutical aspects of fat emulsions.

Although fat emulsions have been used for a long time, their ultimate fate in the body and the mechanisms of clearance have not received much attention. Recently, Boberg, Carlson and Hallberg (1969) have applied pharmacokinetic analysis to rate of clearance of oil particles and the plasma levels of triglycerides.

Linden and Nakayama (1976) have reported the effect of intravenous fat emulsions on hepatic bile and found increased lithogenicity of bile in patients infused with emulsion. Other workers (Jeppsson, 1972; Jeppsson and Schoefl, 1974; Jeppsson, 1976) are investigating the possible use of intravenous emulsions to administer drugs dissolved in the oil phase. Jeppsson (1976) has reported a prolongation of anaesthesia in mice, after intravenous injection of soyabean oil emulsion containing barbituric acid in the oil, as compared with the injection of the corresponding sodium salt solution. The results may be explained by a slow release or by a more specific delivery to the central nervous system when the drug is in an oil droplet. Particles of injected emulsions were observed to adhere to and even to be engulfed by the vascular wall of mammary glands and myocardium. It may be that similar phenomenon applies to the central nervous system, but no such evidence was observed using the same electron-microscopic technique. It is unlikely that fat emulsions can be used to direct specifically a drug to the central nervous system because of the blood-brain barrier, but it could be possible in case of other tissues. An exciting development in this area has been the preparation of liposomes (Gregoriadis, 1974; Ryman, 1974). In these the emulsifier, usually a phospholipid, forms a structured surface layer. It may be possible to incorporate tissue specific antigenic material in this layer so that liposomes will be taken up preferentially by the target tissue. Drugs could be delivered to specific target sites in this way. In recent years much interest has been generated in the use of fluorocarbon emulsions to replace red blood cells.

1.1.3.2 Fluorocarbon Emulsions

1.1.3.2.1 Definition of a fluorocarbon chemical

The term "fluorocarbon" has been used rather loosely by various workers. For example, in anesthesiology the term "fluorocarbon" is used to describe anesthetic agents which contain one or more chlorine atoms. The present definition is based on the one given by Dixon and Holland (1975), and the word "fluorocarbon" will mean a perfluorinated carbon compound which may or may not contain a heteroatom such as nitrogen, oxygen or sulphur but does not contain any other type of halogen or hydrogen atom.

1.1.3.2.2 Some historical aspects

The only fluorocarbons definitely characterized and reported in the literature before 1937 were carbon tetrafluoride, CF_4, hexafluoroethane, C_2F_6, and tetrafluoroethane, $CF_2{:}CF_2$. The main reason for this situation was the difficulties and dangers associated with the preparation and use of fluorine. Moissan, a French Chemist, claimed in 1890 that he had separated CF_4 but his estimation of its boiling point was so grossly incorrect that credit must go to his countrymen, Lebeau and Damiens in 1930, for isolating a sample with boiling point of $-126^{o}C$ ($-128^{o}C$ is the currently accepted value). These workers and Ruff and Keim (1930) also obtained products of higher boiling points than that of carbon tetrafluoride from the fluorination of carbon. Pure hexafluoroethane was first isolated by the Belgian Chemist, Swarts in 1930. Swarts' studies led to the introduction of dichlorodifluoromethane, CF_2Cl_2, by Midgley and Henne, in

America during 1930, as an inert non-toxic refrigerant.

In 1937 the American chemists, Simons and Block, found that mercury promoted smooth reaction between fluorine and carbon (previously the reaction was explosive) at temperatures just below dull red heat. From the reaction products they separated many new, stable, fluorocarbon compounds. From their work emerged the fact that open and closed chains of CF_2 groups are stable and that all the structures associated with saturated hydrocarbons should be capable of duplication in terms of carbon and fluorine.

In 1940 materials were needed for use as buffer gases, coolants, lubricants and sealants in chemical plants handling highly reactive uranium hexafluoride which was the only volatile uranium compound available for use in a gaseous diffusion process for concentrating the ^{235}U isotope required for development of the atomic bombs. It was discovered that a fluorocarbon sample prepared by Simons was inert to uranium hexafluoride. This led to intensive research for methods of preparation of fluorocarbons on an industrial scale and commercial methods for the preparation of fluorine, polytetrafluoroethylene, and polychlorotrifluoroethylene were developed. It was clear from these developments that a new branch of organic chemistry based on fluorocarbons as the parent compounds could be established provided that methods of synthesis could be found for fluorocarbon derivatives.

1.1.3.2.3 Recent developments

In the early 1960's the Naval Research Institute of U.S.A. initiated

the research into screening liquids in order to find those that could dissolve enough oxygen to sustain life. The reason was that it was thought that a liquid breathing deep sea diver would have advantages, such as the ability to reach greater depths, over the conventional gas breathing apparatus in deep sea exploration. Obviously such liquids had to be non-toxic for human use. A typical experiment consisted of immersing a small animal such as a mouse in a beaker full of oxygenated liquid to see if the animal lived.

In 1966, Clark reported immersion of a rat in a beaker full of a silicone oil which had air bubbled through it for a few minutes. The rat lived for almost half an hour before it stopped breathing and died. Later he discovered that fluorocarbon liquids could dissolve larger amounts of oxygen, and repeated the experiment by immersing a mouse into a beaker full of airated perfluoro-butyltetrahydrofuran. After one hour's immersion, the mouse was inverted to drain the liquid from its lungs, and it remained alive. These initial experiments of Clark and co-workers (1966,1971) stimulated much interest and research by many scientists who envisaged the use of fluorocarbon chemicals for many different purposes, which can be divided into two categories, namely for liquid breathing and in emulsion form.

1.1.3.2.3.1 Fluorocarbon liquid breathing

Although it was found that fluorocarbons could dissolve large quantities of oxygen to sustain life, the idea of a fluorocarbon liquid breathing deep sea diver did not become a reality because

later animal studies showed that prolonged breathing of fluorocarbon chemicals caused lesions in lungs. Ruefer (1970) has concluded from his studies that although fluorocarbon liquid breathing does not remove lung surfactants, it does cause an increase in the surface forces. Consequently, a higher pressure would be needed to inflate the lungs than normally. These changes were not normalized within 24 hours after transition to air breathing. However, Gollan and co-workers (1970) have reported the opposite effect occurring with the intact rabbit lungs. They have reported that breathing fluorocarbon fluid "removes" the surface tension at the alveolar-air interface and that, therefore, much less pressure is required to inflate the lungs to the same volume. Perhaps the difference was owing to the use of a different fluorocarbon. However, Gollan et al did find that the large increase in frictional flow resistance of a viscous fluid slowed down its mobility in the airways to a mere trickle. Thus the main problems during a period of liquid breathing are the solubility of gases in the liquid, the kinetics of gas transport in the liquid filling the alveoli, and mechanical factors such as the resistances to flow and friction of the liquid.

The use of neat fluorocarbons was not confined to liquid breathing only. Some workers attempted to use these liquids for organ perfusion as well as liquid breathing experiments. In 1970, Beisang and co-workers reported an attempt to perfuse dog's kidneys with a fluorocarbon liquid. They found it was retained in the kidney tissue and damaged it. The damage was attributed to the higher density of the fluorocarbon. They also reported

that injection of a fluorocarbon liquid into the vascular system was lethal and the intraperitoneal injection had unnoticeable effect, probably because the fluorocarbon does not diffuse through cellular membranes.

The data reported by M.M. Patel and co-workers (1970) indicate that as far as toxicity of liquid breathing is concerned, there is a great deal of difference between the different fluorocarbon chemicals. For example, they found that of the ten hamsters, submerged in one of their fluorocarbons, two died and the remaining eight were bleeding from the nose and mouth, but out of another group of seven, submerged in a different fluorocarbon liquid, two died and the remaining five showed no apparent ill-effects and their behaviour was quite normal during post-submersion survival. These workers have also reported that the histopathologic changes which they observed depended upon the fluorocarbon fluid used and the time interval between removal of the animal from the liquid and its death, but did not depend on the duration of submersion.

In 1970, Spitzer, Sachs and Clark, reported a detailed study of the effects of fluorocarbon liquid breathing on tissue metabolism. They found two apparent effects on in-vivo tissue metabolism, one caused by the lowering of body temperature and a second due to an unknown factor. This second effect, they predicted, was either a general non-specific inhibition of phospholipid synthesis or a specific effect in the synthesis of a common intermediate. The data, reported so far, give no real insight regarding the nature of the decreased metabolism observed in liquid-breathing animals.

Long-term survival of dogs after breathing oxygenated fluorocarbon liquid has been reported by Modell and co-workers (1970). Another use has been the deposition and removal of carbon particles. In 1969 a symposium was held in Atlantic City, U.S.A., on the subject of inert organic liquids for biological oxygen transport. At this symposium many interesting articles were presented on the use of fluorocarbon liquids and fluorocarbon emulsions.

Amongst these were Clark et al (1970) who reported on the physiological effects of fluorocarbon liquids and emulsions. Geyer (1970) reported that the blood of rats could be completely replaced with a suitable fluorocarbon emulsion.

1.1.3.2.3.2 Emulsified fluorocarbons

In 1967 Sloviter and Kamimoto reported in "Nature" that the electrical activity of a rat brain could be maintained by perfusing it with a fluorocarbon emulsion having a particle size range of 2-3 micrometer in diameter. In 1969 Hutson and co-workers found that, compared with natural blood having a haematocrit of 20%, a fluorocarbon emulsion could be 54% more efficient at supplying oxygen to the heart muscle. Subsequently many workers have reported that emulsified fluorocarbons can be used to replace erythrocytes in animals (Clark et al, 1966,1971; Sloviter, 1970). Furthermore, Geyer (1970) has shown that in rats the blood can be completely replaced with a fluorocarbon emulsion. The studies of Clark and co-workers (1970) showed that the fluorocarbon emulsion particles were removed from the circulating blood mainly by the reticuloendothelial system and the platelets. It was clear at

this stage that a detailed study of the colloidal properties of fluorocarbon emulsions was necessary to understand factors which affected the emulsion properties, such as the composition of the interior of emulsion particles, the nature of their coating, surface charge and particle size. By adjusting these factors the emulsion flow properties and clearance from the blood stream may be controlled.

Although satisfactory perfusion of isolated organs such as the liver and whole animals has been achieved by many workers (Triner et al, 1970; Clark et al, 1970) using fluorocarbon emulsions, there were toxic reactions observed (Sloviter et al, 1970). Later Fujita and co-workers (1971) showed that these toxic reactions could be eliminated by making the emulsion droplets small (less than 0.5μ). Furthermore, they found that toxicity was directly related to the particle size. They also reported a method for determining the particle size distribution of a fluorocarbon emulsion, applying Stoke's law of sedimentation. In 1973 Geyer reported further work on complete replacement of blood by a fluorocarbon emulsion and found that rats which had undergone this experiment behaved quite normally.

He also found, as is the case with any colloid, that reducing the particle size increased the viscosity of the emulsion. This imposed a limit on the quantity of a fluorocarbon which could be incorporated in an emulsion to give satisfactory flow properties. This conflicted with the small particle size requirements. Particles much larger than 10 microns, which is the average diameter of erythrocytes,

will not pass through small capillaries and thus increase the risk of embolism. Furthermore, larger particles are removed more quickly from the blood stream than smaller ones. Further investigations of Clark and co-workers demonstrated that after intravenous infusion most of the fluorocarbon emulsion particles were deposited in the liver and spleen, where, depending on the fluorocarbon, they may remain for the life time of the animal or be excreted via the lungs and skin within a few days, without apparent harm to the animal (Clark et al, 1974). The perfluorocarbon chemicals which contain -N- and -O- in the ring structure and straight chain ethers with -O- tend to, somehow, bind to the liver tissue and stay there, but those such as perfluorodecalin and perfluoromethyldecalin are excreted within a few days (Clark et al, 1974). Maugh (1973) has written a review on the possible use of perfluorochemical emulsions as blood substitutes. He concludes that with the discovery of new fluorocarbons which are eliminated from the body within a few weeks the likeljhood of their use as artificial blood substitutes has increased greatly since these are relatively non-toxic compounds.

In 1974 the finding that the elimination rate of those fluorocarbons, which left the liver quickly, was not slowed down when a bromine atom was present in the molecule, stimulated interest in the possible use of iodinated and brominated fluorocarbon emulsions as radiopaque media for the study of circulation (Clark et al, 1974). In 1975, Arambulo and co-workers reported work carried out with perfluorooctyl bromide emulsion as radiopaque media. They found that concentrated (8:1 to 10:1 by volume) stable oil in water emulsions could be

prepared which were non-irritant and useful in bronchography in humans and animals and in angiography in animal studies. Another symposium was held in the U.S.A. in 1974 on the subject of "Artificial Blood" and many papers were presented on fluorocarbon emulsions. Schmolka (1975) discussed the requirements for a suitable emulsifier for artificial blood preparation, and outlined the advantages of Pluronic F-68. He also suggested possible modifications to the Pluronic chain to improve its efficiency as an emulsifier. Fluorinated surfactants were also suggested as "new potential non-ionic emulsifiers". Baldwin (1975) reported that if suitable chelating agents, similar in structure to haemoglobin, could be synthesized in the future and incorporated in a fluorocarbon emulsion it would improve the oxygen release characteristics of the emulsion. Geyer (1975, 1976.) reported further work on complete replacement of blood in rats with a fluorocarbon emulsion, and listed potential uses of an artificial blood preparation (1975). Presenting the "Summary of Workshop" (1975) on artificial blood he concluded that "the demand for blood and its various components is very great" and that "even larger volumes of blood would be used if it were readily available". Surgenor and Mierzwa (1975) concluded that "artificial blood will become important to the extent that it is safer, more effective, more economical and more readily available than natural substances". Most of the remainder of the papers presented at this symposium were concerned with testing of various artificial blood preparations in animals. It was clear that a detailed study of the colloidal properties of these preparations had been neglected and yet the understanding of these properties could be vital to the success

of a fluorocarbon emulsion as a blood substitute.

1.1.3.2.4 Fluorocarbon emulsions as "artificial blood"

The discovery of blood groups by Landsteiner and co-workers has led to the widespread clinical use of homologous blood. The demand for blood for clinical uses is so great that blood from donors cannot adequately fulfill this requirement. Furthermore, blood types are more complex than had been originally thought, and careful typing and matching is required. There is the possibility of transmission of diseases, e.g. serum hepatitis, from donor blood. Another problem is that present procedures only allow the storage of natural blood for a few weeks, hence the need for a blood substitute. The ideal blood substitute should take over the functions of plasma as well as the functions of blood corpuscles, particularly that of red blood cells. Although plasma substitutes have been used clinically for some years, there is still no red blood cell substitute available at present for human use.

Chang (1972) has investigated extensively the preparation and use of artificial cells. One possibility was to enclose haemoglobin solution into artificial cells which would overcome the problem of rapid elimination of free haemoglobin from the circulation. But even haemoglobin solutions do not dissolve enough oxygen to be satisfactory.

Blood is a highly complex fluid having many functions such as transport of oxygen and metabolites to tissues, removal of

carbon dioxide and other metabolic products, maintenance of the concentration of ions and other solutes in extracellular fluids, defence against harmful organisms, etc. Most blood substitutes available at present commercially, perform a few of these functions, but none provides adequate oxygen transport which may be the most critical function of blood, since oxygen lack leads to rapid death.

Recently it has been suggested that emulsified fluorocarbons could find utility as "artificial blood" substitutes having the capability of transporting oxygen and carbon dioxide (Maugh, 1973).

Preliminary studies have been reported by American and Japanese scientists (Clark et al, 1966; Fujita et al, 1971) it is clear from these published data that the success of fluorocarbon emulsions as blood substitutes will be an understanding of the colloidal properties of such systems, for example, their stability, formulation and clearance from the body. These properties are dependent on the physical and chemical characteristics of the fluorocarbon chemical and the emulsifying agent.

Dominant among the properties of fluorocarbons is their relatively high stability, a function of the short interatomic distance between carbon and fluorine and the strength of the bond joining the two. Many emulsifying agents are poorly adsorbed to the fluorocarbon-water interface compared with the hydrocarbon-water interface, consequently it is difficult to prepare a relatively stable fluorocarbon emulsion. Furthermore, for a system to be administered intravenously toxicity is a paramount factor. This

restricts us to the use of relatively non-toxic surfactants. The Pluronics, especially Pluronic F-68, are relatively non-toxic surfactants. Fluorocarbon surfactants also deserve attention, for example, Clark, Kaplan and BeCattini (1970) have reported that some fluorochemical surfactants, which they investigated, have similar or less toxicity than Pluronic F-68 when given intravenously in aqueous 10% w/v solution. Furthermore, on thermodynamic grounds a fluorocarbon surfactant should be optimal for a fluorocarbon oil, because the fluorocarbon chain of such a surfactant is likely to interact more with a fluorocarbon oil than a hydrocarbon chain would interact with a fluorocarbon oil. Consequently there will be greater adsorption of a fluorocarbon surfactant and therefore greater reduction in the free energy of the system giving rise to a more stable emulsion.

1.1.3.2.4.1 Some requirements

Geyer (1975) has listed the requirements which an "artificial blood" preparation should fulfill, and the list of requirements given below is based on this.

A suitable fluorocarbon emulsion should satisfy the following requirements:

(A) Good shelf-life,

(B) good stability in surgical procedures,

(C) no blood group incompatibility problems,

(D) ready accessibility,

(E) no problems with hepatitis, etc.,

(F) low toxicity

(G) no adverse interaction with normal blood,

(H) little or no effect on blood clotting,

(I) satisfactory oxygen and carbon dioxide exchange,

(J) satisfactory rheological properties,

(K) satisfactory clearance from the body. The "ideal" system should be able to take over from the natural blood for a period long enough for new blood cells to be generated, that is it should have a life-time of one to two weeks in circulation before being cleared.

1.1.3.2.4.2 Potential uses

Recently Surgenor and Mierzwa (1975) have published data showing the enormous quantities of human blood, plasma and albumin used in the U.S.A. every year for the various purposes. They came to the conclusion that purely on economic grounds there is a great need for an "artificial blood" substitute. Moreover, artificial blood will be more readily available than the natural substance.

An artificial blood would have many potential uses, some of these are summarised in table 1.1.3.2.4.2-1. This table is based on the one published by Geyer (1975). Fluorocarbon emulsions should be viewed as complementary to blood rather than in competition with it. The emulsions could serve in three different capacities:-

(a) as whole blood, for gas transport,

(b) as plasma, for oncotic pressure,

(c) as erythrocytes, for osmotic pressure and oxygen transport.

It may be possible to include other properties, which the normal blood has, such as clotting, hormone transport, antibody defences.

The various anaemias listed in table 1.1.3.2.4.2-1 all give rise to chronically reduced oxygen carrying capacity of natural blood, for example in aplastic anaemia, it is caused by low red cell production or, in some cases, no red cell production at all. Consequently, people afflicted by these diseases need repeated blood transfusions and often develop intolerance to almost any blood administered. Unless future work proves otherwise, patients would not become intolerant to a perfluorocarbon emulsion. In these cases, an emulsion which remained in circulation for as long as possible would be useful becuase it would minimise the frequency of infusions. The emulsion would maintain an increased pO_2 and therefore markedly reduce the tendency for cells to sickle. This treatment seems better than giving chemicals which directly interact with cells and have to be given repeatedly. It is emphasized here that this is not a cure of sickle cell anaemia, just a new approach to its management.

Table 1.1.3.2.4.2-1 (After Geyer, 1975)

Potential Uses of Artificial Blood Substitutes

1. Anaemias
   (a) Aplastic anaemia
   (b) Sickle cell anaemia
   (c) Cancer-associated anaemia

2. Anaerobic infections, for example tetanus

3. Blood Loss
   (a) Haemorrhage and emergencies
   (b) Surgical operative procedures
   (c) Extracorporeal techniques
   (d) Irreversible shock

4. Vascular Occlusions
   (a) Thrombosis
   (b) Atherosclerosis

5. Toxicity Screening

6. Chemo and Immunotherapy

7. Hormone transport and metabolism; this may become better understood in bloodless state

8. Enzyme depletion and repletion; quantitative studies could be carried out

Table 1.1.3.2.4.2-1 continued

9. Studies on Haematopoiesis

10. Studies on plasma protein metabolism

11. Studies on problems such as sleep

12. Studies on blood-brain barrier

13. New approach to the study of circulatory physiology and haemodynamics

14. Rheology investigation

15. Research on lymph and lymphatics

16. Animal surgery and veterinary medicine

17. Organ perfusion and preservation

18. Total body washout for removal of toxins, viruses, etc.

19. Emergency "blood" transfusion. In National emergency the military use of such a blood substitute cannot be over emphasized.

Anaerobic infections such as tetanus, when not controllable by other means, may respond to the increased oxygen tension that a fluorocarbon emulsion would provide. Because the solubility of oxygen in fluorocarbons is directly proportional to the partial pressure of oxygen, a combination of the hyperbaric chamber and infusion of a fluorocarbon blood substitute would allow very high oxygen tension values than at present attainable with the use of hyperbaric chamber on its own.

In cases of haemorrhage and other emergencies involving large blood loss, blood infusion is needed quickly. Since no blood typing is involved the fluorocarbon emulsion can be given immediately. It would provide both gas transport and colloid osmotic pressure. For these purposes it would be logical to have fully oxygenated emulsions available in emergency vehicles and ambulances. Many surgical procedures and extracorporeal techniques, such as heart-lung machines and kidney machines, require large volumes of blood which can be difficult to provide, in which case an emulsion would be very useful. In case of machines the blood volume required is not only large but much of it has to be discarded after use. Fluorocarbon emulsion could save a lot of natural blood. Furthermore, it could have greater stability to the action of pumps, oxygenators and filters.

Irreversible shock is still not fully understood. Recently a myocardial depressant factor (MDF) has been discovered (Geyer, 1975) which is released into the blood when shock ensues. It is possible that complete body perfusion with a fluorocarbon emulsion would

remove MDF and provide adequate oxygen, oncotic pressure and osmotic pressure which may of itself be helpful in reversing the sequence of events in shock. Subsequently natural blood could be given.

Vascular occlusions give rise to oxygen and nutrient deficits which makes the problem worse. Total perfusion with a fluorocarbon emulsion would not only overcome these problems but also permit the use of a wider range of less expensive clot digesting enzymes. Furthermore, the emulsion could contain vehicles which would remove lipids from atheromas. Bloodless animals could be used to test drugs which normally interact with blood. In the bloodless state a wider spectrum of cells become possible targets for the drug and toxicity testing, therefore, becomes more stringent. Many anti-cancer drugs, e.g. alkylating agents, interact with blood cells or blood proteins, consequently larger doses have to be given in order to get enough drug to the site of action, and these drugs often have a low therapeutic index. Having a bloodless state reduces the necessary dose, and drugs that are attacked by blood enzymes could be given. A similar solution exists with the administration of tumor antibodies.

Hormone transport, metabolism and enzyme studies would be made easier by the use of emulsions for perfusion in-vivo or in-vitro. Pre-existing hormones and proteins could be removed from the circulation and subsequently their concentration may be regulated by the rate of perfusion, and the kinetics of their production may be studied. With no hormones circulating, the introduction

of any given one allows its effects to be investigated without the influence of any other hormones. The same applies to the study of enzymes. The use of protein synthesis inhibitors coupled with total blood replacement would deplete the body of many blood enzymes over an appreciable period of time, following this the renewed synthesis and return into circulation of various enzymes under investigation could be quantitated.

Partial blood removal has been used for a long time in the studies on haematopoiesis and plasma protein metabolism. Artificial blood substitutes make such an approach even more meaningful. As with the hormones and enzymes, the effects of partial or total absence of one or more kinds of blood cells or plasma proteins may be investigated. The rates of synthesis and appearance in circulation can be examined under precisely controlled conditions. The sequence in which different proteins return into circulation starting from almost zero could be followed. If radioactive precursors of the proteins were given at the appropriate time, extremely high specific activities could be obtained in the proteins. Such procedures may have industrial as well as experimental importance. The existence of as yet unrecognised factors may be established.

Investigations of problems such as mechanisms involved in sleep are always carried out with animals having normal blood, cerebrospinal fluid and other fluids. The existence of such factors and whether they travel in blood is not known at present. The "bloodless animal" could be an excellent experimental model to pursue the study of such a factor. Obviously the factors involved may be

restricted to the nervous tissue and the cerebrospinal fluid, even in this situation the bloodless state may be of advantage. There are numerous other examples where the search for humoral or other factors would be aided by the use of the artificial blood substitutes. The full potential of such applications still remains to be explored.

One of the few ways of bypassing the blood-brain barrier (Geyer, 1975) is to increase transiently the osmolality so as to shrink the barrier cells and open up the tight junctions to allow passage of molecules into the brain. This hyperosmolality adversely affects the red blood cells. On the other hand it would have very little effect on a fluorocarbon emulsion. Since this is true of hypo-osmolality as well, a great latitude is possible. It will be necessary, of course, to see if the components of the substitute can also pass into the brain. If they do, their fate and effect will warrant investigation. Probably the limitation of this approach is the overall influence of the hypertonic mixtures on the cells of the blood vessels, for example, sclerosing action.

Partial or total replacement of blood with an emulsion allows the study of circulatory physiology and haemodynamics under new conditions. Variations in viscosity, oncotic and osmotic pressures, buffering systems and flow characteristics can be manipulated within wide limits. The influence of each type of cells on the rheology of blood can be studied by adding each species of blood cell separately. The use of artificial blood combined with techniques such as fibre optics and high speed cinematography

makes visualization within blood vessels possible. A transparent fluorocarbon emulsion would have obvious advantages here. Investigations on lymph and lymphatics can also be aided. The extent to which artificial blood components enter into the lymph is little understood. Lymphocytes continually enter the blood via the lymphatics, and cannulation of the thoracic duct would drain off many lymphocytes. Would animals with lymphocytic leukemia survive longer if totally washed out with an "artificial blood" at the same time thoracic duct drainage is used? A number of interesting experiments could be carried out.

A fluorocarbon emulsion could find use on large scale in animal surgery and veterinary medicine. No blood banks for animals exist and it is unlikely that much progress will be made in establishing them. There are ever-increasing numbers of pets and even riding horses needing veterinary care. Valuable farm animals, rare zoo animals may through accident or illness enter a crisis which requires blood transfusion. A fluorocarbon emulsion would eliminate the species differences involved with real blood transfusions and donor animals would not have to be kept. Information gained in such uses in animals would furnish additional data of importance in considering potential administration to humans.

At present, many organ perfusions are carried out at low temperatures to minimise the oxygen requirement and to decrease the enzymatic activity. It seems self-defeating since the integrity of tissues and cells must rely on the proper functioning of many enzymes. It

would be far better to maintain the organs at regular body temperature, proper nutrient media and conditions of perfusion that would allow almost unlimited survival of them. For this purpose an artificial blood substitute would be advantageous. Furthermore, sufficient natural blood is not usually available for perfusion from the same donor furnishing organ/s. Consequently, incompatibility between the blood used for perfusion and organ is possible. No such phenomenon would occur with an emulsion. Complete replacement of the recipient's blood prior to reimplantation of organ should minimise the chances of organ rejection.

Total body washout to remove toxins, viruses and drug overdose is possible using a fluorocarbon emulsion. At present, oxygenated saline albumin has been used, with some success, in cases of hepatic viral coma. This treatment is of short duration and at lowered body temperature, followed by blood transfusion. With a suitable emulsion longer term body perfusion would be possible at normal body temperature and adequate oxygen availability, thus eliminating the need for a subsequent blood transfusion.

1.1.3.2.5 Toxicity

Much of the work referred to in subsections 1.1.3.2.1 to 1.1.3.2.4 indicates that fluorocarbon emulsions are relatively non-toxic provided they are prepared properly, that is, use of pure chemicals and prevention of fluoride ion production, etc; during preparation. Fluorocarbon chemicals are relatively very stable compounds which is a function of short bond length between carbon and fluorine atoms. The presence of fluorine in the molecule can exert profound influence. For example, in the chlorofluorocarbons

fluorine stabilize s adjacent C-Cl bonds and reduces the steric strain produced by the relatively voluminous chlorine atoms. Furthermore, the accumulation of fluorine atoms on a carbon chain culminates in a changed bond energy and length between carbon and fluorine.

In considering the toxicology of the fluorocarbons it is natural to relate the chemical properties to the biological action resulting from contact with living systems. The stability of the C-F bond is probably the most important consideration and the lack of biological activity could be attributed to it. This can be demonstrated by the fluoroanalogues of the sulphur mustards, $S(CH_2\text{-}CH_2Cl)_2$ and $S(CH_2\text{-}CH_2F)_2$. The chloro compound has a potent vesicant effect which is caused by the dissociation of the covalently bonded, labile, chlorine atom, leaving the highly active halogen-free moiety. It should be noted that the vesicant activity is not due to the released chlorine. The fluoro-analogue, in contrast, is inoffensive owing to the stable C-F bonds which do not easily dissociate.

It is important to realise that there is a clear difference between the properties of fluorocarbons investigated for artificial blood and the "fluorocarbon" anesthetics and propellants which have recently become toxicologically suspect (Ward, 1976; Crawford, 1976; Borchardt, 1976). Indeed, the toxicity of fluorochlorocarbons decreases with increasing fluorine substitution of the carbon chain. The perfluorinated compounds suitable for artificial blood substitutes are biologically, chemically and thermally

inert, Only at very high, pyrolytic, temperatures some dissociation occurs. However, ultrasonic irradiation does cause dissociation of some of the C-F bonds giving rise to fluoride ions (Clark et al, 1975). This is an important factor to remember when preparing fluorocarbon emulsions, for intravenous infusion, by ultrasonication because fluoride ions may be produced in high enough concentration to give rise to a fatal reaction.

1.1.4 The Research Programme

It has long been known that fluorocarbons can dissolve large quantities of oxygen, and when emulsified, could be utilized as artificial blood substitutes. A literature survey was carried out on the work reported on fluorocarbon emulsions as blood substitutes, prior to commencing a research programme on this subject, and updated periodically. A summary of the literature review is presented in table 1.1.4-1.

Table 1.1.4-1 Literature Review

Oil	Surfactant	Reference	Comments
Perfluorotributylamine	Pluronic F-68	Yokoyama et al, 1974	Proposed a method for specifying particle size distribution of fluorocarbon emulsion by means of stepwise centrifugation, based upon Bostock and Stoke's law.
-	A number of fluorocarbon surfactants	Lin, 1972	A modified equation for determining the hydrophile-lipohile balance (HLB) is given.
A number of perfluorinated oils and DC-200 silicones	-	Peterson, 1970	Measured oxygen solubility and other physical parameters such as viscosity, surface tension.

Table 1.1.4-1 continued

Oil	Surfactant	Reference	Comments
Perfluorotributylamine Perfluorodecalin Perfluoromethyldecalin Perfluoro-N,N-di- methylcyclohexylamine Perfluoro-2- isopentylpyran	Egg- lecithin	Yokoyama et al, 1975	Investigated the elimination of fluorocarbons in rats after I.V. infusion of their emulsions and found that all were excreted solely by expiration, except perfluorotributylamine which was also detectable in faeces. Elimination curves were exponential and the rate of each oil was approximately related to its vapour pressure. Perfluorodecalin was recommended because of its rapid clearance from the body as compared with others.
-	-	Baldwin, 1975	The synthetic cobalt-containing oxygen carriers are reviewed as well as some heavier metal systems. Addition of a chelating agent into a fluorocarbon oil has the oxygen release curve similar to that of blood.

Table 1.1.4-1 continued

Oil	Surfactant	Reference	Comments
Perfluorotributylamine Perfluorodecalin	-	Dixon and Holland, 1975	Syntheses of fluorocarbons for potential use as artificial blood substitutes are described. A number of requirements to be fulfilled by such a substance are listed.
-	Pluronic F-68	Schmolka, 1975	Necessary and desirable properties of an emulsifier suitable for an artificial blood preparation are listed and Pluronic F-68 is suggested as a candidate.
Perfluorotributylamine	-	Wallace, Stein and Asher 1975	Studied the feasibility of liquid membrane oxygenation utilizing gaseous oxygen bubbles encapsulated by fluorochemical, thereby avoiding the detrimental changes induced by blood-gas interface.

Table 1.1.4-1 continued

Oil	Surfactant	Reference	Comments
Perfluorotributylamine Perfluorodecalin and other fluorocarbon oils	Pluronic F-68	Clarke et al, 1975	Related some of the physical properties of the oil phases to some of their pharmacological actions and reported that fluorocarbons having vapour pressure greater than about 40 torr must be avoided since they produce pulmonary gas embolism.
Perfluorotributylamine Perfluorobutyl- tetrahydrofuran	Bovine Serum Albumin	Sloviter, 1975	Suggested that fluorocarbon emulsions can be used as artificial erythrocytes to preserve organs such as the brain and kidneys.
Perfluorobutyl- tetrahydrofuran	Pluronic F-68	Padilla, Wear and Wagner, 1975	Reported the effects of fluorocarbon emulsions on the mechanical fragility of normal and sickle cells in vitro. They found no effect in presence of oxygen. In the absence of oxygen normal blood was unaffected but sickle cell blood showed less haemoglobin in plasma when the fluorocarbon concentration was at least 5% in the emulsion.

Table 1.1.4-1 continued

Oil	Surfactant	Reference	Comments
-	-	Surgenor and Mierzwa, 1975	Reviewed "artificial blood" in relation to the national blood policy of the U.S.A. They concluded that "artificial blood will become important to the extent that it is safer, more effective, more economical, and more readily available than natural blood".
-	-	Barker, 1975	Discussed Food and Drugs Administration regulations and licensure and how these may apply to artificial blood.
-	-	Geyer, 1975	Discussed the many and varied potential uses of artificial blood substitutes.

Table 1.1.4-1 continued

Oil	Surfactant	Reference	Comments
Perfluorotributylamine	Pluronic F-68	Geyer, 1975	Reported that in rats the blood can be completely replaced by the fluorocarbon emulsion without any apparent harm to the animals.
Perfluorobutyl-tetrahydrofuran Perfluorotributylamine	Albumin, Pluronic F-68	Nose et al, 1970	Studied the physiological effects of intravenous injection of fluorocarbon emulsion and liquid fluorocarbon chemicals and found emulsion to be much less toxic than the neat liquids.
Perfluorotributylamine	Pluronic F-68	Fujita, Sumaya and Yokoyama, 1971	Showed that the toxicity of fluorocarbon emulsion can be correlated with the droplet size distribution and that reducing the mean droplet size decreased toxicity. They suggested that an emulsion having a narrow particle size distribution with a mean diameter of about 0.2 μm or less would be desirable from the toxicity point of view.

Table 1.1.4-1 continued

Oil	Surfactant	Reference	Comments
Four different fluorocarbons including Perfluorotributylamine and Perfluorodecalin	Pluronic F-68; F.C. 170; Dextran; ASA; ALB;	Clark, Kaplan and BeCattini, 1970	Studied the physiological effects of surfactant solutions and fluorocarbon emulsions. They found Pluronic F-68 to be a non-toxic surfactant and some fluorocarbon surfactants of similar low toxicity. Reducing the particle size of the emulsion caused prolonged dwell time of emulsion in the circulation. They also found prolonged oxygen transporting capability of the emulsion if the blood of the animal was replaced by Ringer's solution and dextran prior to emulsion infusion.

It is clear from the literature review that a vital factor in the success of fluorocarbon emulsions as blood substitutes will be an understanding of the colloidal properties of such systems (their stability, formulation, clearance from the body). In the main the key workers in the field are clinicians and biologists who have little knowledge of colloidal systems. Furthermore, they seem to have no desire to undertake the necessary physicochemical investigations. For example, Geyer (1973/1974) has stated that "if there were centralized source of high quality materials and emulsions, experimenters could simply test the emulsions for physiological effects rather than waste time learning how to produce them". But studies on emulsion systems are far from being a waste of time and would yield vital data that would provide insight into particle stability, and clearance mechanisms.

1.1.4.1 Experimental Programme

In order to provide data on the relevant areas of colloid science a programme was initiated to study the stability of oil in water emulsions and the effect of the nature of the oil phase. The following studies were carried out:

1. Measurement of life-times of single droplets at the plane oil-water interface in order to select emulsifiers which were also good stabilizers and to see if droplet-rest times could be correlated with bulk stability of emulsions.

2. Measurement of surface and interfacial tensions to determine critical micelle concentrations of surfactants and to estimate the extent of adsorption of surface active agents to various

oil/water interfaces.

3. Assessment of the stability of perfluoro-chemical o/w emulsions using particle size analysis (electron microscopy) and viscometry.

It had been reported that if fluorocarbon emulsions are prepared by ultrasonication breakdown of the oil phase can give rise to fluoride ions (Clark et al, 1975). Experiments were carried out to determine the concentration of the fluoride ions. (Fluoride specific electrode) and to see how this varied with the time and intensity of sonication.

Bulk emulsion stability was also judged by accelerated stability tests such as centrifugation and exposure to high and low temperatures. The optimum storage temperature was also investigated.

Any preparation which is intended to be injected intravenously has to be sterile. Therefore, methods of sterilizing the fluorocarbon emulsions were investigated and their effect on the stability of the products, determined.

It has been reported (Yokoyama et al, 1975) that phagocytosis by the reticuloendothelial cells of the liver and other tissues is the main mechanism of clearance of the emulsion droplets from the circulation in the body. Therefore, in vitro phagocytosis experiments were carried out in order to determine some of the factors affecting the rate of phagocytosis.

Oxygen release characteristics of some of the emulsions were determined using an oxygen electrode specially designed for following oxygen release from suspensions.

1.1.4.2 Choice of materials

Sodium dodecyl sulphate as a model surfactant and Pluronic F-68, egg-lecithin, and a number of fluorinated surfactants were investigated. The choice of suitable surfactants is discussed in more detail in subsection 2.2.3. The selection of oils is described briefly in subsection 2.3. Two of these were reported to have poor stability when emulsified but good in-vivo characteristics (Perfluoro-decalin and methyldecalin). Another, perfluorotributylamine has been reported to have good stability when emulsified but poor in-vivo properties. The reagents were chosen as required by the various methods employed.

1.1.5 The Stability Problems

Although the emulsion is a versatile dosage form and has a long history of use as a vehicle for the delivery of drugs, it has never been a pharmaceutical favourite. This has been caused by the inherent, by definition, instability of emulsions. It poses the problem to the formulator of maintaining stability, which means that the appearance of the product is retained as far as is possible, but more importantly, that the emulsion characteristics such as viscosity, drug release, are unchanged during prolonged storage.

The emulsion stability and therefore its properties, are affected

by many factors such as the properties of the oil phase and emulsifier, the particle size, viscosity, disperse phase volume, pH, ionic strength of the aqueous phase. The investigation of these factors is further complicated in the case of multiple emulsions because there are two disperse phases, and consequently, two particle size distributions, two emulsifiers and two phase volumes. The assessment of stability of the larger multiple oil droplets can be accomplished with relative ease by photomicrography to determine particle size distribution and follow the changes in it with storage time, but to size the internal droplets is difficult. Recently, Davis and co-workers (1976)ₑ have reported a technique to size internal aqueous droplets of a multiple emulsion based on electronmicroscopy involving freeze-etching.

The effect of the nature of the oil phase on stability has been investigated in detail by Davis and Smith (1975). They found that the emulsion stability of a number of hydrocarbon oils correlated well with their solubility in the continuous phase and that Ostwald Ripening played an important role. Droplet size increases have been shown to alter the intestinal absorption rate of heparin (Engel and Riggi, 1969) or cause rapid release of antigen from influenza vaccine (Berlin, 1960). Droplet size is very important in emulsions for intravenous infusion, where it should not exceed about 8 microns in order to minimise the risk of embolism.

A direct relationship between particle size and toxicity of fluorocarbon emulsions has been reported by Fujita et al (1971),

who found that a particle size of 0.5 micron or less on average was desirable. Furthermore, emulsions intended for intravenous use require refrigerated storage conditions for maximum stability, and additives such as antibiotics, vitamins and drugs involve high risk of instability.

In research studies one can use a number of oils and surfactants but when it comes to the manufacture of a pharmaceutical product only a few relatively non-toxic surfactants are available.

Changes in the rheological properties of emulsions are particularly important in lotions and creams since the performance and acceptability of these preparations depends on their consistency. Alterations in the appearance of the product is of lesser importance than the changes of emulsion properties in use such as particle size and drug release, although both have to be considered from the point of view of general acceptability of the product. Most important changes in the properties of an emulsion stem from the instability of particle size distribution of a given preparation. This instability involves a number of mechanisms which are discussed in more detail in subsection 1.2.

Vincent (1973, 1976) has reviewed the work done on emulsions, emphasizing the colloidal aspects affecting emulsion stability. S.S. Davis (1974) has discussed the pharmaceutical aspects of fat emulsions. He has stressed that an understanding of the colloidal properties of these emulsions is of fundamental importance.

1.2 Emulsion Stability

1.2.1 Definition

It is difficult to define a stable emulsion because a number of independent processes determine the stability. The classical definition was that there should be "no observable change with time" (Garrett, 1965). This is unsatisfactory since a number of changes may occur such as alterations in particle size distribution which may not be apparent to the unaided eye, but could involve grave risks, in the case of intravenous emulsions, by causing embolism. The stability of an emulsion could be defined as the inverse rate of change of the state of dispersion of the dispersed phase (Groves, 1970). Alternatively, we can say that a physically stable emulsion is one that can be homogenously redispersed to its original state with moderate shaking.

Recently, Carroll (1976) has reviewed the stability and breakdown of emulsions. Generally, the processes causing instability are, flocculation, coagulation, coalescence and molecular diffusion. These processes can proceed independently and simultaneously or can be inter-related. I.U.P.A.C. recommendations for the definitions of these processes are as follows:

An <u>aggregate</u> is an ensemble of two or more droplets held together by forces of unspecified magnitude. Quantizing the interdroplet forces, aggregates comprise <u>flocculated</u> droplets when the forces are weak (interaction energy a few times kT) or <u>coagulated</u>

droplets when the forces are stronger. Flocculates can frequently be redispersed by shaking, while coagulates cannot. Coalescence is the process whereby two droplets unite to give one. Molecular diffusion is a process leading to an irreversible increase in particle size of the largest particles at the expense of the smallest. Its rate depends on the solubility of the disperse phase in the continuous phase and the process is also known as Ostwald ripening. Kelvin recognised the process of molecular diffusion long time ago. The Kelvin equation is given below:

$$RT \ln P_r/P_o = \frac{2 \gamma M}{\rho r} = \frac{2 \gamma V_m}{r}$$

Where R = gas constant

T = absolute temperature

P_r = vapour pressure of droplet of radius, r

P_o = vapour pressure of plane surface of liquid

γ = surface tension

M = the molar mass

ρ = density of the liquid

V_m = the molar volume

r = the radius of the droplet

From this it can be seen that the vapour pressure over a smaller droplet will be higher than over a larger droplet or plane interface of the same liquid. Consequently, there will be more evaporation of liquid from the smaller droplets and greater condensation of liquid over the larger droplets. The result is that smaller droplets get smaller and the bigger droplets get

larger. The term "breaking" of an emulsion covers the two consecutive processes of flocculation/coagulation and coalescence. These processes are schematically represented in Figure 5; Pages 105 -106

When the droplets of an emulsion conglomerate and separate into a layer under the influence of a force the emulsion is said to be creaming. Creaming will occur when an emulsion is placed in a force field that can differentiate the dispersed from the continuous phase. Usually it is gravity but creaming can also occur in electrostatic or magnetic fields. In a gravitational field, if there is a density difference between the two phases, the dispersed droplets experience a vertical force that tends to concentrate them into a layer above or below the continuous phase, according to the sign of the density difference. This tendency is opposed by two ways: temporarily by Stoke's-type viscous forces and continually by the thermal motion (Brownian motion) of the droplets.

1.2.2 Measurement of Stability

Because there is no general agreement on what constitutes a stable emulsion, it is necessary to distinguish several levels of emulsion stability. In particular, aggregation, creaming and coalescence. Accelerated stability tests are frequently employed to predict future stability of an emulsion. These include centrifugation and storage at elevated or lowered temperatures.

Groves (1970) and Sherman (1971) have reviewed the various

accelerated tests that are used to assess emulsion stability. A number of properties may be chosen that can be measured as a function of time, and these will change at different rates (Groves, 1970). The choice of a suitable parameter for the comparison of dissimilar systems has been discussed by a number of authors (Vold et al, 1963; Tingstad et al, 1964; Becher et al, 1965). King (1941) has stated that there was "no single property of an emulsion other than the rate of coalescence or separation of internal phase that can be considered to constitute stability". More recently, Lachman (1970) has suggested that the stability may be measured by the "variation of the distribution of sizes of the dispersed droplets with time".

A number of methods can be used to assess stability and these will be discussed below.

1.2.2.1 Phase Separation

This method is useful for detecting only very unstable systems (Zografi, 1970). The volume of the separated dispersed phase is taken as an indication of stability, the smaller the volume separated, the stabler the emulsion. It is important that the separation is relatively rapid and well defined (King, 1941; Blakey and Lawrence, 1954; Cheesman and King, 1940).

Although the method does not require any elaborate equipment, it is of very little value when it comes to comparing the stabilities of a number of emulsions. Frequently the free oil is difficult to detect initially, or accurately enough to follow the kinetics

of coalescence (Groves, 1970).

1.2.2.2 Particle Size Analysis

Groves and Freshwater (1968) and Walstra et al (1969) have reviewed the methods of determining particle sizes of emulsions. The techniques of microscopy, fluorescence microscopy, photomicrography, Coulter counter and turbidometry have been discussed. Electron microscopy, sedimentometry and surfactant adsorption have also been examined. Sizing of particles larger than a micron is relatively easy and many methods are available but to size submicron emulsion particles, one is limited to only a few methods. Electronmicroscopy, light scattering, centrifugation and low-angle X-ray scattering have been suggested as possible methods to size submicron particles. Recently, Yokoyama et al (1975) have successfully applied a method involving centrifugation and gas chromatography, based on Stoke's law, to size fluorocarbon emulsions having submicron size droplets. A major draw-back of many of these methods, with the possible exception of electron microscopy, is that emulsion dilution is involved which may in itself destabilize the sample and give erroneous assessment of stability.

Particle size measurements are usually expressed as the population within various size ranges and are represented by a histogram or size frequency curve (Sherman, 1968). Berkman (1935) has reported that progressive changes in the size distribution of an emulsion could be followed as a measure of stability. However, Jellinek (1950) has suggested that emulsions of different substances or

those prepared by different methods, will have different size distribution functions (e.g. log normal, skewed, etc.). This point has been further illustrated by Elworthy and Florence (1967, 1969), who found that a comparison was difficult even when size distributions were of similar general form. Nevertheless, size distributions convey a lot of information and therefore are valuable in assessing emulsion stability.

Herdan (1960) has reported that many systems appear to conform to standard distribution functions, especially the normal (Gaussian) or log-normal distributions. Both of these functions can be mathematically re-arranged to give straight line plots (Gaddum, 1945; Davis and Smith, 1973), and the data can be uniquely characterized by only two parameters which are the standard deviation and mean size, (Davis and Smith, 1973). Rajagopal (1959) has given excellent theoretical arguments in favour of emulsion particles conforming to the log-normal distribution. This view has been supported by the experimental results reported by Davis and Smith (1975) and Shotton and Davis (1968). Slight deviations from log-normal distribution have been reported by Rowe (1965) and Hallworth and Carless (1972) for some sodium dodecyl sulphate stabilized emulsions.

A number of functions have been proposed to fit the observed size distributions of emulsions (Groves and Freshwater, 1968; Rajagopal, 1959, Jellinek, 1950). Rowe (1965) has suggested the use of a "polydispersity ratio" for data deviating significantly from the log-normal distribution. It is a ratio of the mass median diameter

to number median diameter (50% of the drops by weight and number respectively that have a value greater than the median). This ratio has been used as a measure of changes in macromolecular stabilized emulsions (Mahrous and Lemberger, 1968).

A number of parameters, e.g. droplet diameter, calculated from the particle size data have been used to express stability in a kinetic fashion. Although the number average diameter is the simplest to derive, the arithmetic, mean surface and mean volume diameters are also useful. To determine the total surface area of a given volume of dispersed phase, volume-surface mean diameter should be measured (Becher, 1965; Sherman, 1968). Sherman (1968) has suggested that the choice of mean diameter should be based on the emulsion property being investigated. Measurement of weight distribution, that is the weight of oil in the various size ranges can also be useful additional information (Mahrous and Lemberger, 1968). Reliance on volume or weight parameters alone can be misleading since these are very sensitive to small numbers of large drops. The reported work of Knoechel and Wurster (1959) showed that over a period of 200 days, measured at 30 day intervals, the arithmetic mean diameter increased progressively with time whilst the volume-surface and weight means showed an initial decrease followed by the increase.

Another popular expression is the specific interface (S), that is the interfacial area per unit weight or volume of dispersed phase. It can be calculated as the area per unit weight from the following equation:

$$S = \frac{6}{\rho_o \, d_{vs}}$$

Where ρ_o is the density of the dispersed phase and d_{vs} the mean volume-surface diameter (King and Mukherjee, 1939). Cooper (1937) found that S was less sensitive to sampling errors than the average diameters such as arithmetic, surface or volume. King and Mukherjee (1939) have found that after initially rapid coalescence the specific interface decreased linearly with time. From this they calculated a stability coefficient which was defined as the reciprocal of the rate of decrease of specific interface. Since then, a number of authors have reported similar results (Jellinek and Anson, 1950; Lawrence and Mills, 1954; Mullins and Becker, 1956).

Hill and Knight (1965) have supported this view by developing a coalescence theory, for uncharged spheres of various sizes. A number of emulsions containing non-ionic surfactants as stabilizers have been found to conform to this theory (Elworthy and Florence, 1967). However, exponential decrease in specific interface with time has been found in other systems such as cottonseed, olive and mineral oil emulsions stabilized by gums (Lotzkar and Maclay, 1943). Another disadvantage of S is its calculation from d_{vs} which is insensitive to very small droplets (those that are outside the range of a given sizing equipment) though these drops do affect the actual interfacial area and emulsion properties (Vold and Groot, 1962).

1.2.2.3 Surfactant Adsorption

A method of calculating total interfacial area, used by some workers, has been based on the analytical determination of the

quantity of surfactant adsorbed onto emulsion droplets. The interfacial area occupied by each surfactant molecule is required, and knowing the total volume of dispersed phase, the average droplet diameter can be calculated. The major disadvantage of this technique is that no information is obtained about the distribution of particle sizes and that the degree of interfacial packing of surfactant assigned to a given emulsion system is subject to error.

1.2.2.4 Droplet Concentration

This method involves the dilution of the sample and counting microscopically the number of drops in a known volume. The results are usually expressed as the numbers of drops into which a unit volume of oil is subdivided, or as the droplet concentration per gram or per cubic cm of emulsion. This method originated from Smith and Grinling (1930) and was modified by Cocktain and Wynn (1952). Van den Tempel (1953) developed a kinetic theory of coalescence based on which he calculated the rate constant from a first order decay of droplet number with time. This approach has been adopted by others (Sri Vastava, 1964; Sri Vastava and Haydon, 1967). Elworthy and Florence (1969) found that rates calculated by this method were more comparable for different systems than size distributions. However, in some instances a non-linear decrease in log droplet number has been reported and consequently the rate constants were difficult to measure accurately and therefore the results were of doubtful significance (Hallworth and Carless, 1972, 1973).

1.2.2.5 Accelerated Testing

Three methods, and modifications thereof, have been generally used for accelerated ageing (Sherman, 1971). These are: exposure to elevated temperature, storage at low temperature and high speed or ultra-centrifugation. Temperature cycling is also used, that is, exposure to low temperature followed by high temperature and so on. It is important that any accelerated test, used for predicting stability, merely speeds up the process causing instability under normal storage conditions. It should not alter this process or introduce any new process.

1.2.2.5.1 Exposure to Elevated Temperature

Usually, a sample is stored in an oven which is maintained at a temperature higher than the normal storage temperature. The volume of the separated dispersed phase or the height of its layer is measured periodically.

Bennett et al (1968) have suggested that "an increase of 10% in the temperature is considered to double the rate of most reactions. Therefore, three months at 45-50^{o}C is equivalent to one year at 20-25^{o}C for many systems". This comment could be true provided it can be shown that the higher storage temperature merely accelerates the mechanism of instability which operates at the lower temperature. However, it is unlikely that heating merely accelerates the mechanism of breakdown, which normally operates, since it lowers the viscosity of the external phase of an emulsion and may also cause discontinuities in the film of emulsifier. The emulsion may invert at a higher temperature

because the solubility of the emulsifier will change in the two phases and it will redistribute itself accordingly. This has been highlighted by Shinoda's work (Shinoda and Arai, 1964; Shinoda and Saito, 1969) on phase inversion temperatures.

1.2.2.5.2 Exposure to Low Temperature

At temperatures below the ambient temperature the solubility pattern of emulsifier can alter. Supercooling of the dispersed phase can occur caused by emulsifiers (Fox, 1959; Skoda and Tempel, 1963). This effect is apparently greater for o/w emulsions than for w/o. Supercooling of 12-15.5°C for w/o and 36.5-56.5°C for o/w have been reported, the value varying with the emulsifier.

Work on refrigerated milk products has led to detailed observations of structural changes which occur when an emulsion is exposed to very low temperatures, but much less is known about the mechanisms involved.

When an oil-in-water emulsion is frozen, water crystals appear pushing the oil droplets into narrowing channels of unfrozen liquid between the crystals. The concentration of electrolytes also increases, supercooling the residual water. The growing crystals force the oil droplets closer together until they coalesce or aggregate depending on the nature of the dispersed phase and the emulsifier. Another important factor is the rate of freezing which affects the recovery of emulsion on thawing.

1.2.2.5.3 Temperature Cycling

This technique involves subjecting an emulsion to alternating high and low temperatures. The usual index of stability is taken to be the number of freeze-thaw cycles a product can withstand before it cracks, under a standardised procedure. The factors involved are as yet poorly understood, but probably involve a combination of the features of both high and low temperature conditions. The test is more realistic from a practical point of view since the product would normally be stored at fluctuating temperature.

1.2.2.5.4 Centrifugation

Many workers have reported the use of the centrifuge technique to study emulsion stability. In the dairy industry it has been used at low speeds (3600 rev/min., Merrill, 1953) to accelerate creaming to measure the accelerated rate of separation of the dispersed phase. Others have used higher speeds (20000 rev/min.) and found that logarithm of particle number decreased linearly with time, (Cocktain and Wynn, 1952). However, most emulsions are too stable to be affected by this relatively low stress and therefore ultracentrifuge has been used (Vold and Groot, 1962, 1963,1964 , 1964 ; Garrett, 1962; Rehfeld, 1962). The method only provides information on the coalescence of the process, since the volume of oil separated is usually taken as a measure of stability. The emulsion under centrifugal stress is under a different physical state from a system under gravitational stress, especially at the ultra-centrifuge speeds. It is therefore unlikely that centrifugal method would give realistic ageing

behaviour of emulsions under normal conditions, especially if the flocculation process is the rate-determining step in the degradation of the product under normal storage conditions. However, Mittal (1975) has reviewed the work published on the ultra-centrifugal technique in the study of emulsions and has concluded that it could be a useful method and has the advantage of requiring less time.

1.2.2.6 Single Droplet Coalescence at a Plane Interface

This method involves measuring the "coalescence times of single droplets. The denser phase is usually placed first in a container and the lighter phase layered on top of it, the surfactant being dissolved in one of the phases. A single drop is formed of one of the phases, usually oil phase, which either rises or falls depending on the density and comes to rest at the interface where it stays for a finite time before coalescence with the bulk phase. A number of drops are measured and a range of coalescence times is obtained. Generally, the longer the drops take to coalesce the stable the emulsion should be. Exceptions to this rule have been found, but the test is roughly quantitative and useful for detecting gross differences in emulsion stability at room temperature. A more detailed account of this method is given in subsection 3.2.

1.2.2.7 Other Methods

Measurement of the viscosity of an emulsion is another way of estimating its stability. Because the rheological properties are sensitive to particle size changes it could be a good method

of detecting early changes in the particle size distribution of an emulsion. However, there are many factors which influence the emulsion viscosity by altering the droplet size distribution and therefore it is necessary to study in detail how droplet size affects viscosity.

Parkinson, Matsumoto and Sherman (1970) have reported their work with monodisperse suspensions of Polymethyl methacrylate in Nujol. They investigated the effect on viscosity by blending together these suspensions over a size range of o.1 to 4 μm to get different modal diameters. Groves (1970) has suggested that the degradation of some emulsion systems could be accelerated by shearing them in a concentric cylinder viscometer of the Couette type or in a cone-plate arrangement such as in the Ferranti-Shirley viscometer. The main problem here would be to assess the degree of change or instability. In the case of emulsions used for cutting or rolling of metals, the failure to lubricate, which will increase the friction, may constitute a valid end-point. But since sample sizes available are usually small, measurement of particle size and change thereof after shearing stress may be more practicable. The apparent viscosity under applied Shear stress is a complex function of particle size and factors such as flocculation state. Therefore it should be possible to combine rheological measurements with acceleration of instability by shearing stress.

'A study of the dielectric properties of an emulsion system can be utilized to provide information on the internal structure and properties of emulsions. This method has been extensively reviewed

by Hanai (1968).

The disadvantage of this method was that the cell capacitance of the measuring cell was only sensitive to dielectric charges in emulsions of low conductivity and could not be used for emulsions containing ionic emulsifiers.

Jackson and Skauen (1962) found that the electrophoretic mobility of o/w emulsions prepared from cottonseed oil, brominated cottonseed oil, sesame oil and brominated sesame oil showed some relationship to emulsion bulk stability on storage at both room temperature and 40°C. Electrophoresis studies provide information about the surface charge of particles which affects the flocculation rate rather than the coalescence rate. If flocculation was the rate determining step only then these measurements would have some relevance to the prediction of stability.

1.2.3 Maintenance of Stability

Emulsion stability towards coalescence or breakdown is generally conferred by the accumulation at the interface of certain types of substances which give rise to a large energy barrier in the coalescence "reaction path" (droplet separation, potential energy or V-H curve). These substances were referred to as the "third component" in the definition of an emulsion. Disregarding certain inorganic compounds, which act only feebly (Cheesman and King, 1940), the stabilizers most commonly used are:

(a) Amphiphilic organic compounds such as the detergent molecules. These contain a hydrophobic and a hydrophilic

part. The hydrophilic moiety may be ionic or it may ionize at the interface, or be nonionic.

(b) Macromolecules such as proteins, synthetic polymeric compounds, and polyelectrolytes.

(c) Solid particles which are partially wetted by both phases.

The exact mechanism and the nature of the energy barrier produced by stabilizing agents is not fully understood. It will depend on the chemical nature of both adsorbate and the adsorbent (Florence and Rogers, 1971). The stabilizer may:-

(i) Decrease the free energy of the system.

(ii) Form a physical interfacial barrier between droplets.

(iii) Affect the electrostatic charge on the surface of the droplets.

1.2.3.1 Stabilization by Surface Active Agents

A surface active agent may be stabilizing an emulsion by one or more of the following effects:

1.2.3.1.1 Decrease in Free Energy

The adsorption of a surface active agent at the oil-water interface reduces the interfacial tension. This leads to decreased interfacial free energy of the system. This effect alone may not be sufficient to produce stable emulsions since coalescence could still occur (Becher, 1962). It has also been reported that emulsions having the same interfacial free energy may exhibit different stabilities (Florence and Rogers, 1971). Lawrence (1952) has stated that a markedly low interfacial tension may even cause instability due to the reduced tendency of droplets

to remain spherical. It is now generally accepted that a reduced interfacial tension is indicative of adsorption of a surfactant but not necessarily its stabilizing effect. Stability depends on the formation of a "rigid" interfacial film which can prevent coalescence.

1.2.3.1.2 Steric Stabilization

After two emulsion particles have approached closer than the separation corresponding to the potential energy maximum (DLVO theory, appendix I), they tend to draw together under the influence of Van der Waal's forces until short range forces (Born repulsive forces) become dominant. The process with two drops is qualitatively different because at small separations some flattening of the drops occurs. A thin circular film of continuous phase separates the two drops. This film has a small but finite radius which reduces the drainage rate of the continuous phase from between the drops as compared with two rigid particles. Coalescence of the droplets occurs when the film ruptures.

In steric stabilization the presence of the emulsifying agent forms a physical barrier which enables the droplets to approach each other but not close enough so that the potential energy maximum is passed. Thus droplets can lie in close proximity of each other without coalescing. The stability of the emulsion does not merely depend upon the "strength" of the film formed by the stabilizer. It is the current opinion that the barrier represents a combination of steric, viscous and elastic properties which depend upon the properties of the emulsifier (Florence and

Rogers, 1971). The stability depends on the ability of the film to withstand distortion and displacement and to reduce the drainage of the continuous phase. A rigid film may not be able to withstand small localized defects in the interfacial region. This view has been supported by the work of Elworthy et al (1971) who found that hexadecanol enhanced the stability of chlorobenzene emulsion stabilized with a nonionic surfactant (hexa oxyethylene glycol monohexadecyl ether), but hexadecanol alone gave poor stability even though its film was more rigid.

Earlier studies on adsorbed films (Fischer and Harkins, 1932; Bancroft, 1915; Harkins, Davies and Clarke, 1917) concerned with an explanation of emulsion type and inversion, regarded the film as purely a mechanical barrier. Stabilization by finely divided solids and some macromolecules has been assumed to be due to the rigidity of the film (Kitchener and Mussellwhite, 1968). With the possible exception of mixed films, it is unlikely that the stabilizing effect is purely due to the mechanical rigidity of the adsorbed film, particularly in the case of ionic surfactants. It has been postulated (Davies and Rideal, 1961) that a "solvation barrier" may also exist against coalescence due to loosely held water molecules.

The most important properties of the interfacial film are its permanence and coherence. It is thought that many macromolecules form a viscous, strong, elastic film (Kitchener and Mussellwhite, 1968; Sri Vastava, 1964; Cumper and Alexander, 1950; Blair, 1960). A viscous film will have reduced drainage rate, an elastic film

will be able to withstand distortion due to collision of droplets and a closely packed film will have general mechanical strength. All these properties are desirable to obtain maximum stability. However, the role played by interfacial viscosity in stabilizing an emulsion is poorly understood mainly because it is difficult to measure the relevant properties directly using the presently available equipment (Becher, 1965 ; Kitchener and Mussellwhite, 1968). There is a group of workers who are of the opinion that interfacial viscosity is not significant in emulsions stabilized by macromolecular surfactants in general (Blakey and Lawrence, 1954). Others have found that increased interfacial viscosity was accompanied by enhanced stability (Carless and Hallworth, 1968; Hallworth, 1971), but there were exceptions. Sherman (1973) has stated that more detailed rheological studies are required using more advanced techniques so that the coalescence mechanism is better understood.

Recently Napper (1977) has written an excellent review on steric stabilization discussing the various theories. The author also stipulates the properties of the best steric stabilizers. He states that amphipathic block or graft copolymers provide the best stability. The most effective of these consist of both anchor groups and stabilizing moieties. The stabilizing moieties must be soluble in the dispersion medium to be effective whereas the anchor groups function most effectively if nominally insoluble in the dispersion medium. The purpose of the anchor part is to prevent the stabilizing moieties attached to one particle from moving away from the interaction zone on the approach of a second particle. The stabilizing moieties could in principle escape from

the stress of the interaction zone by two different mechanisms. First, by lateral movement around the surface of the particle while remaining attached to the particle, or secondly, in some circumstances, desorption may occur from the particle surface. Lateral movement could be prevented by fully coating the surfaces and desorption could be overcome by attaching the stabilizing moieties to suitably insoluble polymer chains that anchor, either chemically or physically, the moieties to the particles. Stabilizing moieties not anchored or poorly anchored can impart some stability to colloidal dispersions but these flocculate and stabilizer displacement or desorption does occur.

1.2.3.1.3 Electrostatic Stabilization

Many years ago Lewis (1910) realised that an electrical charge on the surface of emulsion droplets could stabilize an emulsion since like charges repel. It was thought that particles could become charged by adsorption of ionic substances, ionization of chemical groups, or friction. The adsorption of ionic substances such as surfactants has been pictured as the polar "head" groups protruding through the interface into the aqueous phase and the hydrophobic parts "tail ends" penetrating into the hydrophobic environment of the interior of the oil droplets. Ionization of the adsorbed groups could surround the droplets with a charge which would impart stability by keeping droplets apart from each other. Davies and Rideal (1961) and Powney (1941) have reported that in the absence of a surfactant droplets of par affinic oils carry a negative charge. This charge could be due to adsorption (or desorption) of polar groups such as hyroxyl at the interface.

Alternatively, the charge may be caused by friction which would tend to make the oil phase negative relative to the higher dielectric aqueous phase (Coehn's rule, Coehn, 1898).

(A) The Electrical Double Layer

Since in an emulsion there will be both negatively and positively charged ions, any concentration of one type of ions will cause accumulation close to them of ions of opposite charge (counter ions). Thus, these ions will form a parallel double layer. Because the counter ions would be mobile the double layer will be of a diffuse nature, the charge density of the layer decreasing exponentially with distance away from the droplet surface. Stern (1924) combined the models of double layer of Helmholtz and Gouy into a two-part double layer. The inner part consisting of an attached layer of counter ions (Stern Layer) having a sharp potential difference across its thickness and the outer layer having gradual fall in potential being analogous to the Gouy diffuse layer.

(B) Double Layer Repulsion

The significance of the electrical double layer in emulsion stability is that it gives rise to repulsive forces between particles which may prevent coalescence of emulsion droplets. Derjaguin and Landau (1941) and Verwey and Overbeek (1948) have attempted to quantify these forces. The DLVO theory, named after these authors, is an evaluation of the total attractive (Van der Waals) and repulsive (electrical) forces between the two particles. A more detailed description of the DLVO theory, its limitations,

and modifications are given in Appendix 1.

The experimental evidence is conflicting regarding the importance of the electrical double layer in emulsion stability. Limberg (1926) found no correlation between droplet mobility in an electric field, measured by moving boundary technique, and emulsion stability. Similarly, King and Wrzesz inski (1940) found no relationship between electrophoretic mobility (a measure of droplet surface charge) and stability to coalescence. Florence and Rogers (1971) and Hallworth and Carless (1972, 1973) have concluded that in many cases the stability cannot be fully explained on the basis of VT (total interaction between two particles - DLVO theory, dependent on electrical charge). On the other hand there is evidence that the DLVO theory does provide a reasonable description of the electrostatic barrier towards aggregation (Lawrence and Mills, 1954) and that electrical forces make a definite contribution to stability if the interfacial film was neither mobile nor readily desorbed (Elworthy and Florence, 1967; 1969).

1.2.4 Nature of the Interface

In addition to the obvious dependence of the structure of the interface on the physical properties of the adsorbed film, the chemical nature and viscosity of the oil phase are important factors affecting emulsion stability.

1.2.4.1 The Effect of Oil Phase

Relatively fewer investigations have been reported on how the nature of the dispersed phase affects emulsion stability. King (1941) has reported marked differences in stability of emulsions

prepared with the same emulsifier (sodium oleate) but different oils and mixtures of oils. It was found that the density of the oil phase was important in determining stability but the viscosity could not be related to bulk viscosity of emulsion or stability. The chemical nature of the oil phase was also important, for example, a high polarity gave poor stability of nitrobenzene emulsions.

It would be logical to postulate that the nature of the oil phase will affect significantly the properties of the adsorbed film of emulsifier. The work of Toms (1941) and Shotton and White (1960, 1963) shows that the chemical nature of the oil phase determines the extent of interaction between the oil phase and the surfactant and therefore the structure of the adsorbed film, which in turn influences the emulsion stability. It was found that emulsions of light liquid paraffin were more stable than those of benzene, while those of cyclohexane were intermediate, using acacia as emulsifier. The stability differences were attributed to the variation in the thickness of the adsorbed film with the type of oil. The same effect has also been reported with non-ionic surfactants (polyoxyethylene glycol hexadecyl ethers) (Florence and Rogers, 1971).

Garrett (1965) has stated that the effect of the nature of the oil phase on emulsion stability is negligible, except in the case of highly polar oils. Bernstein et al (1971) showed that initial coalescence rate (less than 3 hours ageing) in highly dilute emulsions of dioctyl or dibutyl phthalate and hexadecane,

containing low concentrations of sodium dodecyl sulphate, was independent of oil phase. However, there is some evidence that the oil phase does affect emulsion stability irrespective of the polarity of the oil phase (Groot, 1965). Further evidence comes from Schulman et al (1959) and Prince (1970) who have prepared microemulsions and have shown that the stability is highly dependent on the oil phase. Furthermore, Davis and Smith (1975) have shown that addition of small amounts of an oil, which forms stable emulsions, to an oil which forms relatively unstable emulsions, enhances the stability of the emulsions prepared from the mixture. The exact mechanism of this stabilizing effect is not fully understood. The concept of "required -HLB" for a given oil is further evidence of the dependence of stability on the nature of the oil phase. The HLB (hydrophile - lipophile balance) is affected by the chemical nature of the continuous phase, dispersed phase and the emulsifier. Each oil could be ascribed an optimum HLB value for maximum stability of a given emulsion system (Becher, 1965 ; Sherman, 1968 ; Griffin, 1954; Gorman and Hall, 1963).

Consequently, different dispersed phases will give different stabilities, being stabilized by the same emulsifier.

1.2.4.2 Adsorbed Layers at the Liquid-Liquid Interface

Coalescence of drops happens when the interfacial film ruptures. Therefore, the properties of this film are very important for emulsion stability. Adsorbed layers at liquid surfaces can be divided into two basic types. If the molecules of the adsorbed

film are insoluble in both dispersed and continuous phase, they have to be spread on to the interface by some means and the resultant film is called "spread" or "insoluble". Relatively little work has been done on such films at liquid-liquid interfaces because of the experimental difficulties involved (Jones et al, 1963; Brooks and Pethica, 1965; Taylor et al, 1973; Taylor and Mingins, 1975). Surface layers formed by adsorption of materials which are soluble in one or both of the "solvents" are termed soluble or adsorbed films. The "solvents" in studies of liquid-liquid systems are usually chosen so that they have low mutual solubilities and for this reason, alkane-water and benzene-water systems have been popular.

Another interface of interest would be that between a liquid mixture and an immiscible liquid. Very little work has been done on this (Aveyard, 1967).

The extent of adsorption is usually estimated from interfacial data using the Gibbs adsorption equation (details of derivation of Gibbs equation are given in appendix 2). In using the Gibbs equation, it is often assumed that activity can be replaced by concentration of the adsorbate because, usually, only very dilute solutions are involved. However, especially for adsorption of hydrogen bonding adsorbates from non-polar solvents, serious deviations can arise owing to autoassociation of the adsorbate molecules. Neglect of these factors can lead to erroneous calculations, (Aveyard, Briscoe and Chapman, 1973).

Wetting and adhesion are also important in adsorption at interfaces.

The cohesion between identical molecules and adhesion across the interface may be defined as follows (Becher, 1965 ; Sherman, 1968).

The work of cohesion (Wc) is defined as the work required to split, laterally into two units, a cylinder of liquid of unit cross-sectional area. It is given by:

$$Wc = 2\gamma_2$$

where γ_2 is the surface tension of liquid. Similarly if two systems are in contact, these may be separated to give two new surfaces and the work required for this is called work of adhesion (Wa) given by:

$$Wa = \gamma_1 + \gamma_2 - \gamma_{1,2}$$

Where γ_1 and γ_2 are the tensions of the two new surfaces formed and $\gamma_{1,2}$ is the interfacial tension between the two liquids.

When a drop of liquid 1 is placed on a liquid substrate 2, it may remain as a lens or spread across the surface of 2, depending on whether Wa is greater than Wc. Harkins (1952) introduced the idea of spreading coefficient to quantify this phenomenon. The spreading coefficient, s, is defined by the following equation:

$$s = Wa - Wc$$

$\therefore s = \gamma_2 - \gamma_1 - \gamma_{1,2}$ where γ_1, γ_2 and $\gamma_{1,2}$ are measured as mutually saturated phases. Therefore, spreading will occur if Wa>Wc.

1.2.4.2.1 Physical States of Films

Harkins (1952) and Adamson (1967) have extensively discussed the states which could be ascribed to films at interfaces. A summary will be presented here.

(i) Gaseous films.

A gaseous film is said to be formed when the area per molecule of adsorbate is much greater than the molecular cross-sectional area. It is assumed that in such a film there are no predominant intermolecular forces between the adsorbed molecules.

(ii) Liquid films.

In a liquid film there is some interaction between the adsorbed molecules, but the area per molecule is still significantly larger than the cross-section. This is taken as an indication that the molecules are loosely packed at the interface. Liquid films can give two forms of force-area curve, depending on whether they are expanded or condensed. Liquid expanded films show a limiting area per molecule of about 0.5 nm^2 (Langmuir, 1933) for adsorbates having straight chain molecules. These films on compression show a sharp transition into an "intermediate" type of film which consists of small islands of condensed-liquid film in equilibrium with expanded liquid film. Further compression leads to a liquid condensed film, containing tightly packed polar "heads", as a result of rearrangement of adsorbate molecules and elimination of water molecules out of the film. This state of the film is characterized by having a linear force-area region of low compressibility. The limiting area per molecule can be as low as 0.25 nm^2. Alexander (1941) has considered the structure of liquid condensed films. More recently, Aveyard and Vincent (1977) reviewing liquid-liquid interfaces have discussed liquid films in detail.

(iii) Solid films.

The characteristic of these films is that the force-area graph is quite linear. The film has low compressibility. The limiting area to which these films may be compressed approximates to the area of vertically packed chains of the molecules. Typically these films are formed by long chain fatty acids and alcohols on water surfaces.

1.2.4.2.2. Behaviour of Adsorbed Layers

It is well known that the nature of a bulk gas can be deduced indirectly by observing the way in which its pressure varies with volume at constant temperature. In a similar manner the state of adsorbed and spread species has often been inferred from the way in which the surface pressure, π varies with the area, a, occupied by each molecule of adsorbate. It is apparent from the Gibbs equation that positive adsorption must lead to lowering of γ from its value (γ_o) for the clean interface. π is defined as $\gamma_o - \gamma$. A surface equation of state, which relates π and a, has the general form:

$$\pi = RT\ f\ (a)$$

Where f is a mathematical function, R, the gas constant and T, the absolute temperature.

1.2.4.2.2.1 Surface Equations of State

The free energy of the adsorbate at an interface depends on the state of the adsorbed film and, for soluble films, the way in which the adsorbate is distributed between the bulk solution and the surface is therefore of interest. The mathematical

expression which relates bulk activity to the interfacial concentration, at constant temperature, is called the adsorption isotherm, and may be written as:

$$a = k\, f'(\Gamma)$$

Where Γ is the surface excess of adsorbate (moles/litre) and k is a proportionality constant containing the standard free energy of adsorption. The relationship between k (which is basically a distribution coefficient for the partition of adsorbate between bulk and interface) and the standard free energy of adsorption is analogous to the Vant Hoff isotherm.

For a given system, the adsorption isotherm and equation of state $[\Pi = RT\, f\,(a)]$ are interconvertible via the Gibbs adsorption equation (De Boer, 1968).

It has been assumed sometimes that surface equations of state are analogous with equations of state for bulk systems. However, it would be better, using a suitable model system, to derive an equation from first principles by, say, the use of statistical thermodynamics. As far as suitable model systems are concerned, liquid-liquid interfaces may be taken as being homogenous. Most adsorbed and spread layers are likely to be monomolecular and non-localized (that is the molecules can translate freely within the plane of the surface). Although the adsorbate is mixed with one or both of the "solvents" at the interface, it would be simpler to treat the adsorbed layer as a one component surface phase. Alternatively, a more realistic approach would be to treat it as a two-dimensional solution, (Vincent and Aveyard, 1977).

1.2.4.2.2.2 Adsorbed Layers of Simple Nonionic Substances i.e. Non-polymeric

Haydon and Taylor (1960) have shown that adsorbed layers of various non-ionic surfactants, including the shorter chain n-alkanols (C_2 to C_{10}), at the petroleum ether-water interface obey the Volmer equation. The equation can be tested by rearrangement to get:

$$\frac{1}{\pi} = \frac{a}{RT} - \frac{a_o}{RT} \quad \text{----- The Volmer equation.}$$

Thus, if the equation is applicable, $\frac{1}{\pi}$ plotted against, a, should give a straight line graph of slope $\frac{1}{RT}$ and intercept of a_o at $\frac{1}{\pi} \simeq 0$. Subsequently, Aveyard and co-workers (1972 and 1973) have confirmed the findings of Haydon and Taylor. They found that the value of a_o/N_A was independent of chain length for the n-alkanols, and equal to about 0.25 nm^2 $molecule^{-1}$, (Haydon quoted a value of 0.18 nm^2 per molecule). Pal and co-workers (1975) have shown that monolayers of various unionized mono and dibasic alkanoic acids at the benzene-water and heptane-water interfaces also obey the Volmer equation.

Although the Volmer equation is applicable to monolayers at the oil-water interface, it does not fit the air-water interfacial data. A possible reason for this could be the lateral cohesion between the hydrophobic groups at the air-water interface. This also means that for a given area per molecule, the π value is lower than that for oil-water interface, the difference being the "cohesive pressure".

However, the difference in the π value may not be purely due to cohesion between the molecules. Since alkyl chain molecules, at

high values of, a, are likely to lie flat along the air-water surface, a_o/N_A would be different from the value for the oil-water interface. The larger value of a_o/N_A for the air-water interface would give rise, according to the Volmer equation, to a larger value of π. Therefore the cohesive pressure as defined above is probably an underestimate of the reduction in the value of π, due to lateral attraction between adsorbate molecules.

Even though the Volmer equation is relatively successful, one would expect that an equation based on a two-dimensional solution model be even better. Fowkes (1964) has reported the, apparently successful, use of an equation similar to the Volmer equation. Aveyard and co-workers investigated in more detail to see if this equation could be applied. They found it was not possible to calculate a, and a_2 independently from the best fit of π, a, data, but that it was necessary to fix the value of a, based on the assumed orientation of the interfacial solvent molecules. On the assumption that the adsorbate molecules were mixed only with the alkane molecules at the interface (not with water), the π, a, data for alkanols could be fitted quite well. But the π, a, curves for films of esters of dicarboxylic acids were not reproducible. Therefore, we may infer that the Volmer equation is useful as a fitting equation but not suitable for obtaining information about the physical realities. However, it is surprising that the two-dimensional gas model should apparently be better than the solution model. Obviously there is a need for further modifications to obtain a more realistic theory for such systems.

1.2.4.2.3 Polymer Layers

Very few studies of the configurational and dynamic properties of polymers adsorbed at liquid-liquid interfaces have been reported. Much more work has been published for solid-liquid interfaces (Lipatov and Sergeeva, 1974; Stromberg, 1967; Rosoff, 1969; Ash, 1973).

Lankveld (1970) has reviewed the literature on the effect of polymers on interfacial tensions in liquid-liquid systems. The use of Gibbs adsorption equation to derive the adsorption isotherms from interfacial data is questionable for such systems because the adsorption is irreversible. Nonetheless, Katchalsky and Miller (1951) have considered the theory of the reduction, $\Delta\gamma$, in interfacial tension resulting from polymer adsorption. This theory incorporates the Gibbs equation and agrees well with experimental findings. The authors predicted a linear relationship between $\Delta\gamma$ and log (polymer concentration).

Lankveld and Lyklema, (1970, 1968 and 1972) have considered the steady state and the time dependent effects of polyvinyl alcohol on liquid paraffin-water interfacial tensions. An unusual effect which they found was that in the case of polyvinyl alcohol samples containing a low (about 2%) acetate content, there was a discontinuity in the plotts of interfacial tension versus log of polymer concentration. The reasons for it were given to be the changes in the relative rates of diffusion of polymer molecules to the interface and configurational rearrangements at the interface. Bohm and Lyklema (Bohm, 1974; Bohm and Lyklema, 1972 and 1975)

found similar results with adsorbed polyelectrolytes at the liquid paraffin-water interface.

When a solution of two polymers in the same solvent undergoes phase separation, a somewhat different type of interface is formed. In this case, the same solvent species is present on both sides of the resultant liquid-liquid interface. Theoretical discussions of such systems have been given by Silberberg (Silberberg and Kuhn, 1952, 1954) and more recently by Helfand (Helfand and Tagami, 1971 and Helfand and Sapse, 1975).

1.2.4.2.4 Charged Layers

Much work has been published investigating the theory of electrical double layer. Both soluble and insoluble ionic monolayers, usually consisting of long chain ions, formed at the hydrocarbon-water interface to minimise lateral cohesion, have been used. The presence of charge would cause lateral repulsion between the adsorbed molecules leading to an increase in the surface pressure. Recently this effect was demonstrated experimentally by Pal et al (1975). The ionization also causes an increase in the free energy of the monolayer. Therefore, in the case of soluble monolayers, the extent of adsorption is reduced relative to that for the uncharged analogue. The double layer theory can be used to predict the magnitude of these effects, and conversely the study of charged monolayers can be used to test the theory.

2. MATERIALS

2.1 Water

Double-distilled water from an all glass apparatus comprising of two stills connected in series was used in all experiments. The first litre or more of the distillate was discarded to reduce any atmospheric impurities and the remainder collected in glass aspirators which were stoppered when full. The water was stored for a few hours and its surface tension measured which was taken as an indication of its purity and was always 71 to 72 mNm^{-1}, at $25^{o}C$ measured by the Wilhelmy plate method. The Conductivity of the water was also measured and it was always less than 1.2×10^{-6} Ω^{-1} Cm^{-1}.

2.2 Surfactants

2.2.1 Surfactants Investigated

Table 2.2-1 lists the surfactants which were investigated and their suppliers.

Table 2.2-1 ; Surfactants

Surfactant	Supplier
Monflor 51) Monflor 52) Monflor 53)	Imperial Chemical Industries
F.C. 126) F.C. 170) F.C. 176) Forafac 1111) Forafac 1023) Forafac 1032) Pluronic F-68)	3M United Kingdom Ltd. BASF - Wyandotte Corporation
Egg-lecithin Sodium Dodecyl Sulphate	British Drug Houses Ltd.

2.2.2 Characterization

Very little information was available from the manufacturers about the Monflor, FC., and Forafac series of emulsifiers. These were relatively new compounds and only a few were commercially available. The basic chemical structure of these fluorinated surfactants is a stable fluorocarbon tail (in the Monflor series this is highly branched) and a solubilizing group X, $CF_3 (CF_2)_2$ ....X. The solubilizing group can be organic or inorganic, anionic, cationic, nonionic, water soluble or oil soluble. The surfactants were characterized by the determination of molecular weight, critical

micelle concentration (cmc), and for some of the surfactants micro-analysis to find out the elements present in the molecule and their percentage. These physical parameters have been tabulated in table 2.2-2. The molecular weight was determined in most cases by mass spectroscopy, the critical micelle concentration was calculated from surface tension data (subsection 4.1). The lecithin was also characterized by its Rf value (0.68) using thin layer chromatography.

Table 2.2-2 : Parameters of Surfactants

Surfactant	Molecular Weight	Elements Present in the molecule and their Percentage					Critical micelle concentration (Molarity)	Water Solubility (g/litre) at 25°C
		C	H	N	S	F		
Monflor 51	560	45.62	6.29	-	-	48.09	1.0×10^{-4}	> 100
" 52	470						-	< 10
" 53	309						-	< 1
F.C. 126	393						8.0×10^{-5}	∿ 80
" 170	140						8.2×10^{-4}	∿ 100
" 176	554						-	insoluble
Forafac 1111	887.43	40.33	5.18	-	-	54.49	7.0×10^{-5}	> 250
" 1023	893	27.48	1.46	2.42	-	68.64	4.0×10^{-4}	0.4
" 1032	914	20.26	1.50	2.46	5.92	69.86	1.2×10^{-3}	> 250
Pluronic F-68	8350						-	∿ 100
Egg-lecithin	802							
SDDS	288.38						8.1×10^{-3}	∿ 60

2.2.3 Selection of Suitable Surfactants

Since the fluorocarbon emulsions are intended to be used as artificial blood, the emulsifier or emulsifier system (a mixture of two or more surfactants can be used) must meet several requirements for both human and animal use, (Schmolka, 1975).

A. Obviously, it must be nontoxic and must not contain or develop on ageing any toxic impurities.

B. It must be soluble in physiological saline at a pH of 7.4.

C. It should be chemically inert, that is, it must not form complex compounds with any component of natural blood. It must not cause haemolysis of erythrocytes or interfere with normal function of the various components of blood e.g. clotting factors.

D. It must be relatively stable to oxygen and carbon dioxide.

E. It must be readily excreted from the body.

F. It should function efficiently as an emulsifier, that is, only a small concentration should be sufficient to produce stable emulsions.

The following are additional desirable but not absolutely necessary requirements.

G. It should be commercially available with high purity and in large quantities.

H. It should be inexpensive.

I. It should function as a plasma expander to reproduce the oncotic pressure normally provided by blood proteins.

J. It should be utilized by the body as a source of energy.

K. It should not support growth of micro-organisms.

None of the surfactants investigated fulfilled all the requirements listed, but Pluronic F-68 fulfilled most of them. It is a non-ionic surfactant, nontoxic at low concentrations, LD_{50} is more than 10 grams per Kg of body weight (Green Cross Corporation literature for "Flusol-43"). Unlike all ionic and many nonionic surfactants it does not cause haemolysis of erythrocytes.

Pluronic F-68 has a mean molecular weight of 8350 and produces stable emulsions. Recent clinical and experimental studies have shown that it may alleviate some of the adverse effects of extracorporeal circulation. When added to the circulating blood in a final concentration of 0.6 mg/ml it has been reported to reduce fat embolization (Adams et al, 1959; Clark & Gollan, 1966) haemolysis (Miyauchi et al, 1966; Paton et al, 1968; Robinson et al, 1973) and sludging (Geyer, Monroe & Taylor, 1968 ,) as well as lower viscosity without changing the hematocrit. These investigators are almost ready to accept the use of Pluronic F-68 for extracorporeal circulation and to allow it in whole circulation of humans. Therefore Pluronic F-68 was the obvious choice for further investigations.

Egg-lecithin was also chosen because of its non-toxicity and emulsifying efficiency. It has been used widely for preparing fat emulsions used for intravenous nutrition (Intralipid contains 1.2% egg lecithin as emulsifier, Davis (1976)). Recently, Yokoyama et al (1975) have used it successfully to prepare fluorocarbon emulsions. However, it has the disadvantage of supporting bacterial growth, which was minimised by its storage at low temperature under acetone after purification.

Sodium dodecyl sulphate (SDDS) was chosen to act as a reference for comparing the emulsifying and stabilizing efficiency of the various surfactants investigated. It is a well characterized substance obtainable in highly purified form and a lot of work has been reported on its use.

A number of fluorinated surfactants were chosen because it was thought that these would be more efficient emulsifiers of fluorocarbon oil phase since the fluorinated chain of such a surfactant could interact with the oil phase more than a hydrocarbon chain; (the energy of transfer of $-CF_2-$ is probably less than that of $-CH_2-$ from aqueous to a perfluorinated oil phase).

2.2.4 Purification

Since Pluronic F-68 and Egg-lecithin were intended for intravenous use their purity is of paramount importance. As obtained from the manufacturers, the egg-lecithin contains impurities of pigments and lysolecithin and Pluronic F-68 contains hydroquinone derivatives as antioxidants. Lysolecithin can give a fatal reaction by causing haemolysis. Furthermore, for interfacial studies the purity of substances is very important. Therefore Egg-lecithin, SDDS and Pluronic F-68 were further purified. The remainder of the surfactants (fluorinated surfactants) were used as obtained from the manufacturers because insufficient information was available regarding their chemical structure thus making purification difficult.

2.2.4.1 Egg-lecithin

90 g. of egg-lecithin was dissolved in as small a volume of chloroform as possible and placed on an alumina packed column (Alumina type 'H'

Laporte Industries). The column was about one metre long and 5 cm. in diameter. It was eluted with 6:1: : Chloroform:methanol to remove impurities such as pigments. Then it was eluted with 2:1: :Chloroform:methanol. All the lecithin was in this eluate. The organic liquids were removed by evaporation under vacuum. The purified lecithin was stored at $4^{o}C$ under acetone.

Thin layer chromatography was used to check the purity of the lecithin particularly to see if any lysolecithin impurities were present. Two solutions, in chloroform, were prepared, one containing purified lecithin and the other lysolecithin. Each solution was applied as a separate spot on a silica gel thin layer chromatography plate. It was run in methanol:chloroform:water: :6:14:1 mixture. The plate was developed by spraying with Dragendorf's reagent, after drying, and the Rf value of lecithin and lysolecithin calculated. The Rf value of lecithin was found to be 0.68 and that of lysolecithin 0.51. The formula for Dragendorf's reagent is given in table 2.2.4-1.

Table 2.2.4-1 : Dragendorf's reagent

Solution A	
Bismuth Subnitrate	17 g
Tartaric acid	200 g
Distilled Water	800 ml
Solution B	
Potassium Iodide	160 g
Water	400 ml

Equal volumes of solutions A and B were mixed prior to use as developing reagent for the thin layer Chromatography Plates.

2.2.4.2 Sodium dodecyl sulphate (SDDS)

A sample of 0.1 Kg. of SDDS was recrystallized from a 50% v/v ethanol-water mixture. The surfactant was extracted using heptane over a period of about 35 hours in a Soxhlet apparatus. The surfactant samples were washed with fresh solvent and dried under vacuum.

The purity of the samples was tested by gas-liquid chromatography. About 1 g. of the surfactant sample was refluxed for four hours with a mixture of 30 ml water and 10 ml hydrochloric acid. The mixture was cooled and the free alcohols extracted using ether. The ether was evaporated and a solution prepared in heptane containing about 10 mg per cm^3. of the free alcohol. This solution

was chromatographed using Perkin-Elmer F17 instrument with a flame ionization detector. The carrier gas was nitrogen with a flow rate of 1 ml per second. The column used was Antarox CO-990 packed with 8% Antarox on 80-100 mesh Chromosorb W AW - DMCS (Perkin Elmer Ltd.) The Column was operated at 184^{o}C. Pure dodecanol was used to find retention time and the purified sample was compared with this. It was found to be 99.2% pure.

2.2.4.3 Pluronic F-68

The method used for purification of Pluronic F-68 was based on the method developed by Yokoyama and Co-workers (Personal Communication) 20 g. of Pluronic F-68, industrial grade, were dissolved in 100 ml of ice cold water (the solubility of Pluronic F-68 decreases with increase in temperature and vice-versa.) The resultant solution was passed through a column of Cation "Amberlite IR 120" and anion "Amberlite IRA 400" exchange resin. In order to remove hydroquinone derivatives which were present in the flakes of Pluronic F-68 as antioxidants, 0.5 to 0.8 g of activated Charcoal were added to the Pluronic F-68 which had been treated with ionic exchange resin. The solution was finally filtered with a millipore membrane (0.45 micron in pore size) and the resultant solution was used as the purified Pluronic F-68. It could be lyophilized for storage if necessary.

2.3 Oil Phase

All perfluorinated chemicals were obtained from Bristol Organics Ltd., Bristol, and n-hexane was purchased from British Drug Houses Ltd. Six Perfluorocarbon chemicals were studied. Perfluorodecalin, Perfluoromethyldecalin and perfluorotributylamine were chosen for

further investigation because it has been reported that perfluorotributylamine gives relatively stable emulsions but poor *in vivo* characteristics (that is, it is deposited in the body for a long time) whereas the Perfluorodecalins form emulsions of poor stability with good *in vivo* characteristics, that is, the clearance rate from the body is relatively fast (complete elimination in 2 weeks).

2.3.1 Purification

All the perfluorinated compounds were further purified by distillation under reduced pressure and their purity determined by gas-liquid chromatography as described in subsection 3.3.2.2.1. Table 2.3-1 lists the purity of each chemical as determined by G.L.C. (gas-liquid chromatography).

n-Hexane was purified further as follows: The oil was washed with fuming sulphuric acid until no rapid colourization was observed. It was then washed twice with water, twice with 5% w/v sodium bicarbonate solution and finally five times with distilled water. Activated charcoal and fused calcium carbonate were added and the mixture left to stand overnight. The oil was filtered, distilled under vacuum and finally passed through fresh activated alumina columns until the oil-water interfacial tension reached a maximum.

Table 2.3-1 ; Purity of oils

Oil Phase	Purity (% by GLC)
Perfluoro tributylamine	98
" decalin	95
" methyldecalin	97
" hexane	99 (85% n-isomer)
" methylcyclohexane	97
" 1,3-dimethylcyclohexane	97
n-hexane	> 99

2.3.2 Characterization

The oils were characterized, after purification, by measuring their physical properties such as boiling point, density, surface tension and relative viscosity. These characteristics are listed in table 2.3-2 for each oil investigated.

Table 2.3-2 : Some Chemical and Physical Constants of oils

Oil Phase	Boiling Point °C	Density g/cc at 25°C	Surface Tension mNm^{-1} at 25°C measured	Surface Tension mNm^{-1} at 25°C reported	Relative Viscosity at 25°C	Molecular weight	Molecular formula
Pf. tributylamine	176-177	1.890	16.31	16.1	6.003	671.13	$C_{12}F_{27}N$
Pf. decalin	142-143	1.931	18.63	-	6.198	462.11	$C_{10}F_{18}$
Pf. methyldecalin	160-161	1.972	20.10	-	6.703	512.12	$C_{11}F_{20}$
Pf. hexane	57	1.694	11.97	11.88	0.794	337.97	C_6F_{14}
Pf. methylcyclohexane	76	1.808	14.24	14.2	1.841	349.97	C_7F_{14}
Pf. 1,3-dimethylcyclohexane	102	1.848	14.91	15.0	2.019	399.97	C_8F_{16}
n-Hexane	68-69	0.658	18.43	18.43	-	86.18	C_6H_{14}

2.4 Miscellaneous Reagents

All other chemicals such as Ethanol, Chloroform, Sodium Chloride etc. were analar grade. Benzotrifluoride and 111-trichlorotrifluoroethane used in gas-liquid Chromatography were obtained from Bristol Organics Ltd. and used without any further treatment. The culture media used in the phagocytosis experiments and in the sterility tests were purchased from Oxoid Ltd.

3. METHODS

3.1 Surface and Interfacial Tension

3.1.1 Choice of Method

The various methods for measuring surface and interfacial tensions have been reviewed by Padday (1969).

The surface tensions were measured by the Wilhelmy Plate method incorporating an electronic microforce balance. It is quick and easy to operate and ageing effects can be followed. The atmospheric contamination was minimised by having the relevant parts of the apparatus enclosed in a chamber.

The interfacial tensions were measured by the pendant drop method. This method was chosen for its higher accuracy. It incorporated a camera arrangement which was used to take a picture of the pendant drop. The drop profile was determined from the image. The accuracy of this method is about $\pm$ 0.1% (Padday, 1969).

3.1.2 The Wilhelmy Plate Method

3.1.2.1 Principle of measurement

The force, F, acting on a wetted plate hanging with its lower edge in and parallel with the plane of the surface of a liquid is given by

$$F = mg = 2(l + l_0)\,\gamma$$

where m is the apparent increase in the weight of the plate, g the acceleration due to gravity, l and l_0 are the length and the end correction of the plate respectively, and γ is the surface tension of the liquid. The factor 2 is there because there is a meniscus of liquid on both sides of the plate.

3.1.2.2. Preparation of Plates

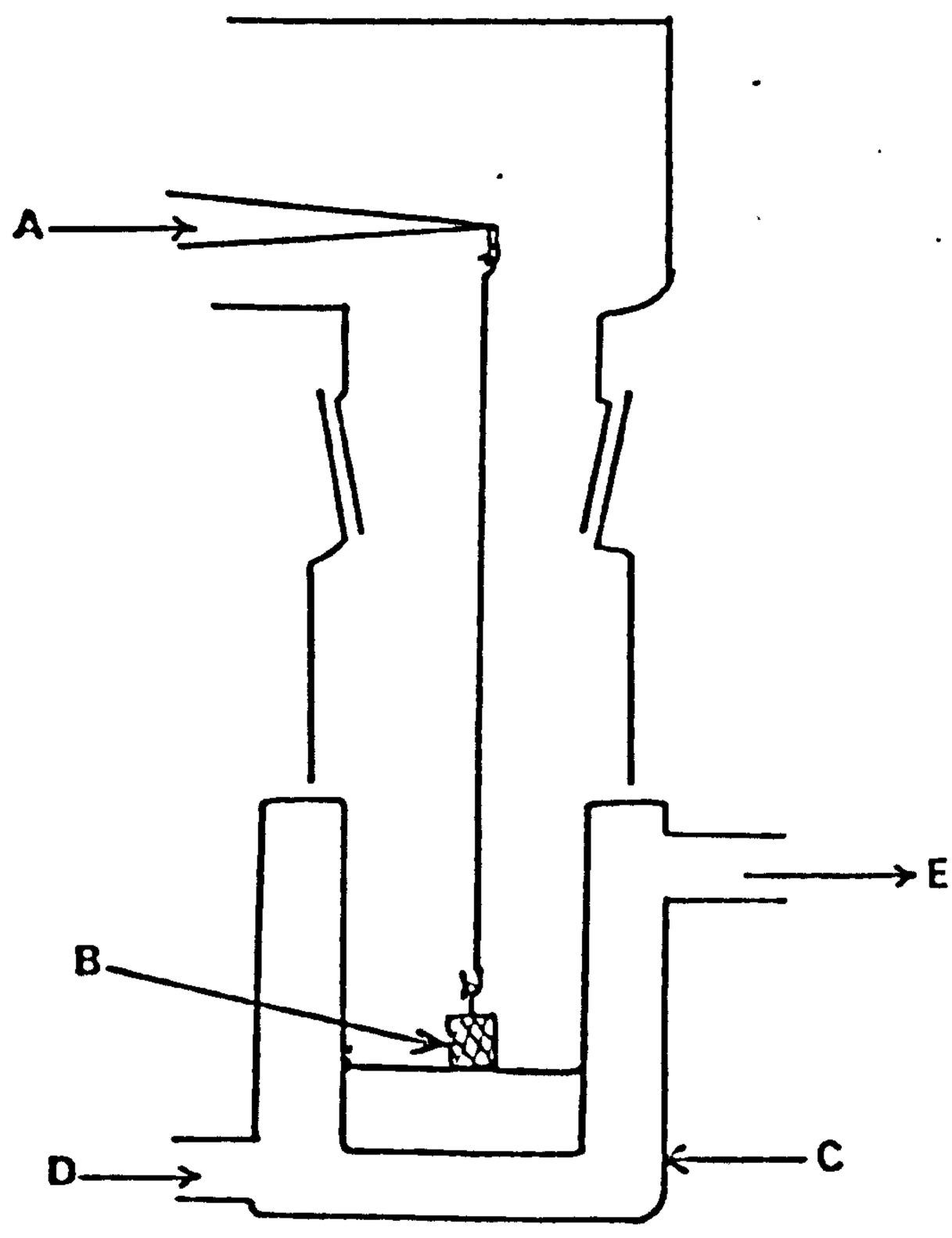
The plates were cut from a precision strip of polished platinum (Johnson and Matthey Ltd.). A hook of platinum wire (3.3×10^{-2} cm. diameter, from same supplier) was silver soldered onto the upper edge of each plate. The plates were roughened by sand blasting to improve wetting. The plate dimensions were measured with a travelling microscope. Before each surface tension measurement the plate was cleaned by heating in a bunsen flame and cooled.

3.1.2.3 Operation

An electrical microforce balance (Beckman RIIC Ltd; LM 800) was used to measure the apparent increase in weight of the plate (i.e. m, in the equation). The balance was equipped with the surface tension measurement accessory, (see Fig.1). An x-y chart recorder was used in conjunction with the balance to provide higher accuracy. The balance was calibrated, according to the instructions in the manual, with accurate known weights (supplied by Beckman Ltd.), before surface tension measurements were carried out.

The plate was cleaned and returned to the balance before each measurement and the zero position of the balance re-checked. 20 ml of test liquid at $25^{o}C$ was placed in a double walled thermostatted beaker maintained at $25^{o}C$ by pumped water from a water bath. The beaker was positioned on the platform and raised just below the plate. The image of the plate in the liquid was used to check that the edge of the plate was in parallel with the liquid surface. The beaker was raised further and as soon as the plate touched the liquid it was dragged in. The beaker was lowered so as to make the

Figure 1 Showing the Principle of the Wilhelmy Plate method



A -- Side arm of Electronic microforce balance

B -- The Wilhelmy Plate

C -- Double jacketed beaker

D & E -- Water inlet and outlet respectively from the thermostated waterbath.

lower edge of the plate stationary, in parallel and within the surface of the liquid. The mean of five readings was taken as the apparent increase in weight of the plate.

3.1.2.4 Determination of the End-Correction

The end correction is approximately equal to the thickness of the plate but due to roughness of the plate this cannot be accurately measured by direct method. Therefore 3 or 4 plates were made of the same height and thickness but different lengths (length of the edge parallel to the liquid surface). Each plate was suspended from a hook and weighed first dry and then in contact with a pure liquid (carbon tetrachloride). The end correction was obtained by plotting apparent increase in weight against the length of the plate. Extrapolation to zero increase in weight gave the value of l_0, the end-correction.

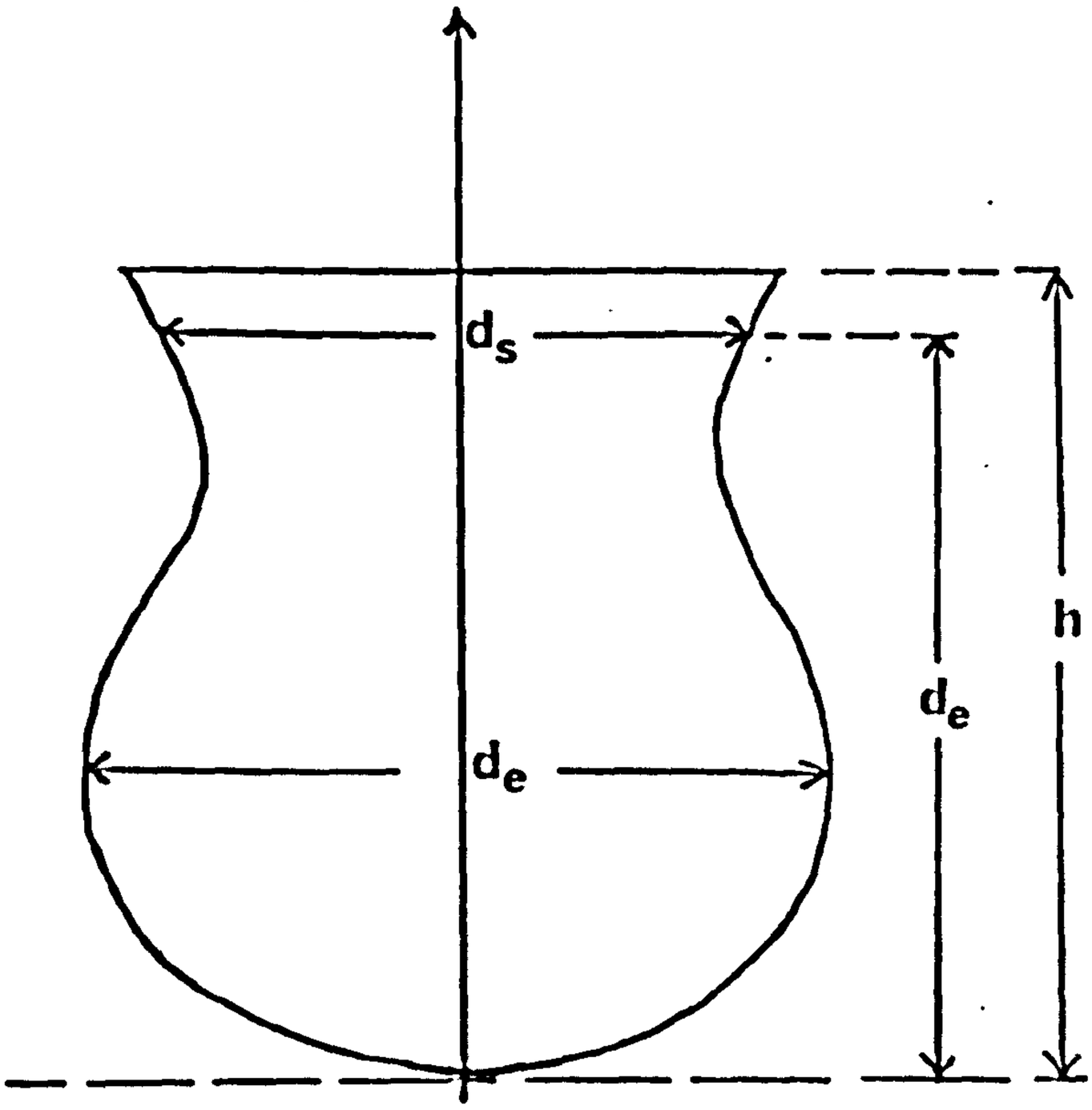
3.1.3 The Pendant Drop method

Interfacial tensions were measured by the pendant drop method. This method is particularly suitable for measuring interfacial tension between two liquids because the drop is never detached from the capillary tip and thus it is a true equilibrium method.

3.1.3.1 Principle of measurement

Pendant drop menisci are completely described by the equations of Bashforth and Adams (1892). Hence, it is possible to measure interfacial tension accurately knowing two related pendant drop parameters (d_e and d_s see Fig 2).

Figure2 Pendant drop Profile



where

de is equatorial (maximum) diameter

ds is diameter at a distance of de from the bottom of the droplet

h is droplet height

Andreas, Hauser and Tucker (1938) showed that a ratio, S, derived from d_e and d_s was related to H which was related to the interfacial tension, γ_i, as follows:

$$S = d_s/d_e$$

$$H = -\beta(d_e/b)^2 = \rho g(d_e)^2/\gamma_i$$

Where β, b, ρ and g have the same meaning as in the Bashforth and Adam's treatment.

Knowing S, H was obtained using tables of Fordham, (1948) or Stauffer (1965), depending on the value of S. The relevant tables are given in appendix 3. γ_i, was calculated using the equation given above, and a mean of four measurements was taken as the interfacial tension between the two liquids concerned.

3.1.3.2 The Apparatus Arrangement and Operation

The apparatus consisted of a transparent perspex chamber the inside atmosphere of which was thermostatically maintained at 25°C. Inside the chamber was a double walled beaker, maintained at 25°C by circulating water, having an optical window made of special glass to minimise distortion of the pendant drop. The less dense liquid was placed in this beaker. The pendant drop forming equipment in the chamber consisted of a jacketed metallic holder through which heated water was circulated to maintain the temperature (25°C) of the contents of Agla syringe held inside the metallic holder by means of a screw. The syringe was operated by a micrometer gauge so that the volume of the drop could be accurately measured if desired. The syringe was fitted with a detachable teflon capillary tip of suitable ratio of the

bore diameter to the capillary tip outer diameter.

The denser liquid was placed in the syringe and the 'lighter' in the beaker. It was allowed to attain thermal equilibrium for 30 minutes. The capillary tip was immersed in the liquid in the beaker and pendant drops were formed and allowed to age for five minutes. Pictures of pendant drops were taken on 35 mm film (Panatomic X, Kodak) using a camera and stand (Nikkormat FT2, Nikon, Japan). The camera had bellows attachments to take close-up pictures of pendant drops.

The image of the drop on the negative was magnified many times on a shadowgraph (to reduce % error in measurement), and the drop profile determined (d_e and d_s) accurately. From the drop profile data the interfacial tension was measured.

3.1.4 Interfacial Adsorption

Interfacial tension data were collected over a wide range of surfactant concentrations. The area (A) occupied by each surfactant molecule at the interface was calculated using the derived Gibbs surface excess equation. For dilute solution,

$$\Gamma = -\frac{C}{RT} \cdot \frac{d\gamma}{dc}$$

where Γ = excess concentration of solute per unit area of surface.

C = concentration of surfactant.

R = gas constant.

T = absolute temperature.

$d\gamma/dc$ = the rate of increase of the surface (or interfacial) tension of the solution with the concentration of the solute.

According to the equation a negative $d\gamma/dc$ implies a higher concentration of solute at the surface than in the bulk of the solution.

At sufficiently dilute concentrations it may be assumed that solute-solute interactions at the surface are negligible and the film obeys ideal two-dimensional gas equation, $\Pi A = kT$

where Π = surface pressure

A = Area

k = Boltzman Constant = R/N_A

T = absolute temperature

Furthermore the lowering of surface tension will be approximately linear with concentration

i.e. $\gamma = \gamma_0 - bC$ where b is constant

$\therefore \Pi = bC$ and $d\gamma/dC = b$

substituting into Gibbs equation

$$\left(\Gamma = \frac{-C}{kT} \frac{d\gamma}{dC} \right)$$

where Γ = molecules in excess/unit area

and k = Boltzman Constant

gives $\Gamma = \frac{1}{A} = \frac{\Pi}{kT}$

$\therefore \Pi A = kT$

and $A = \frac{1}{\Gamma}$

A more detailed derivation of the Gibbs adsorption equation is given in appendix 2.

3.2 Droplet Stability

A number of workers have found an overall general correlation between single droplet stability and bulk emulsion stability (Silber & Mizrahi, 1975; Davis et al 1976, Jeffreys & Davies, 1971). The data obtained can be analysed in a number of ways which are discussed in more detail in subsection 3.2.3.

The stability, that is the rest times, of single droplets at a plane interface can be affected by many variables such as vibration, temperature, density difference of the two phases, volume of the droplets, distance the drops fall under gravity before resting on the interface, hydrodynamic effects, etc. Many of these variables can be held constant and only then different systems may be compared. For example, temperature and volume of the drops can be fixed, and so can the distance which the drops travel before reaching the interface, this distance should be at least about 10 times the diameter of the droplets which ensures that the droplets have reached their terminal velocity before reaching the plane interface and thus all the drops will hit the interface with the same force. The density difference can be taken into account when calculating the stability parameters.

Therefore the single droplet stability may be useful as a general guide to bulk emulsion stability, but it should be remembered that there may be important exceptions. The method may also be useful generally to screen various surfactants to select the ones which will give better stability.

3.2.1 The Coalescence Cell

An all glass cell was made on the basis of the design by Nielsen

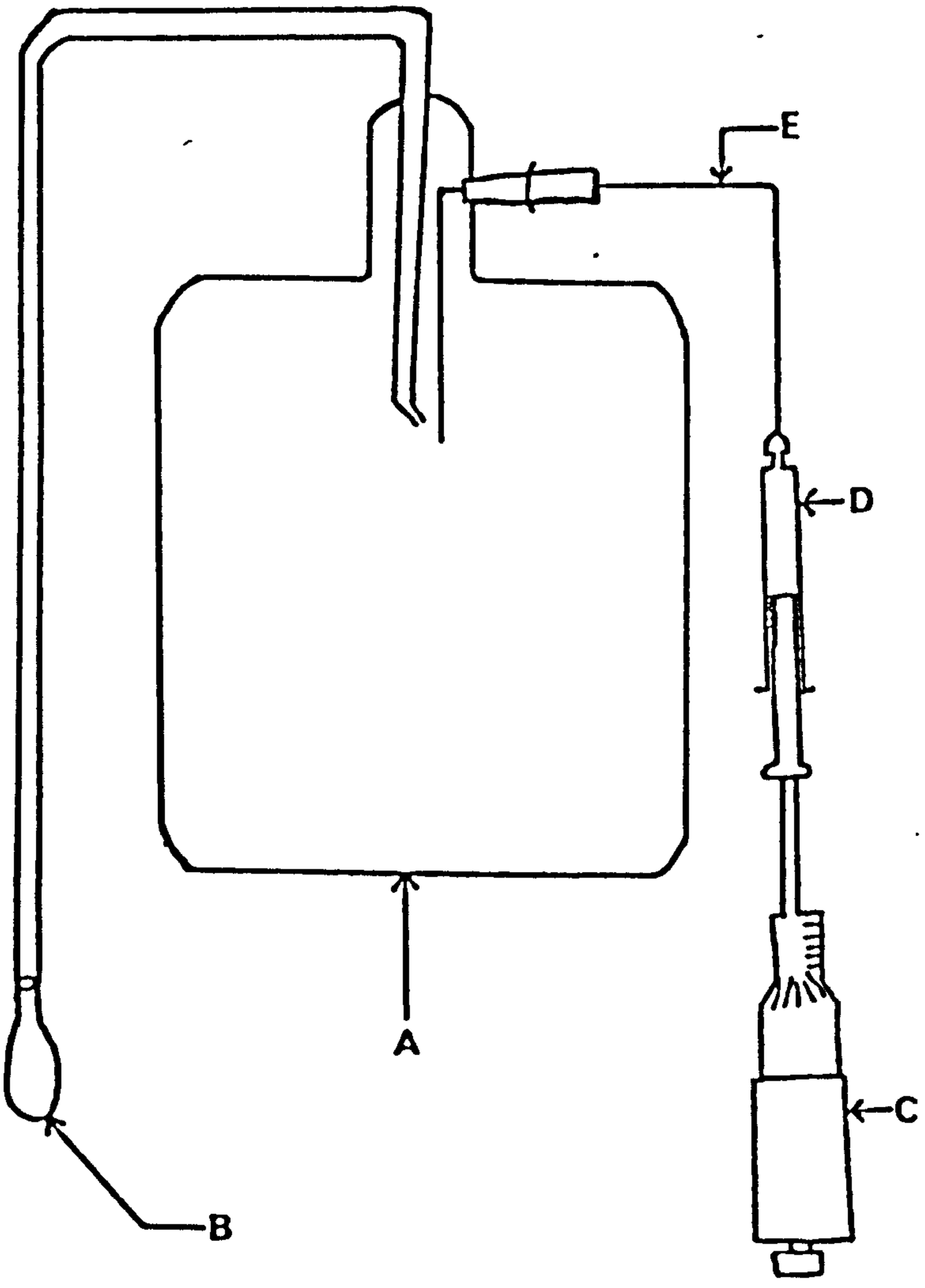
et al (1958) with some modifications (see Fig 3). The cylindrical chamber of the cell was 10 cm internal diameter. Oil droplets of equal volume were formed singly at the tip of a filling canula (A.R. Horwell Ltd.) by means of an 'Agla' micrometer syringe (Luer fitting, Wellcome Reagents Ltd.). Access for the canula was provided near the top of the cell through a socket into which fitted a glass cone of the appropriate size. The canula was sealed into the glass cone with 'Araldite' (Ciba-Geigy). Provision was made for droplets of the selected volume to be released from the canula tip by squirting liquid through a small nozzle situated immediately above the canula tip. The liquid was squirted simply by gently pressing a teat fitted onto a capillary continuous with the nozzle.

3.2.2 Operation

The glass cell was cleaned with chromic acid, rinsed thoroughly with distilled water and then rinsed with the appropriate surfactant solution. The 'Agla' syringe and canula arrangement was rinsed thoroughly with the organic phase before filling the syringe with the organic phase.

The cell and the syringe arrangement were assembled and clamped into position. 500 ml of the aqueous phase was added into the cell and 50 ml of the organic phase. The whole arrangement was allowed to equilibrate to 25°C in a thermostatically controlled water bath (Townson & Mercer Ltd.). After thermal equilibration, the oil droplets were formed and aged at the canula tip, then detached. The rest-times (time from when the droplet hit the interface to when it coalesced with the bulk of the oil phase) of droplets were measured by a stopwatch. At least 80 droplets were timed for each

Figure 3 Showing the arrangement of the Single droplet coalescence apparatus.



A -- Coalescence cell

B -- Rubber teat

C -- Micrometer gauge

D -- Agla Syringe

E -- Metal Cannula

different oil phase and each experiment was repeated. Satisfactory reproducibility was obtained.

3.2.3 Expression of Droplet Stability

Davis & Smith (1973) and Jeffreys & Davies (1971) have reported obtaining a distribution of droplet rest-times. Similarly, Silber & Mizrahi (1975), using orange oil and gum arabic, have reported obtaining a distribution of single droplet rest-times at the oil-gum solution interface. A number of stability parameters can be obtained by statistical analysis of these data. For example, Cockbain & McRoberts (1953) utilized a graph obtained by plotting log. N (the numbers of droplets remaining at time t) versus t for a number of organic phases. The aqueous phase had a surfactant dissolved in it. The initial slow decrease in N was attributed to film drainage from between the droplet and the interface. The subsequent, more rapid and almost exponential part of the graph enabled the calculation of a rate constant for coalescence.

$$\text{Log } N = (-k_c t/2.303) + \text{constant}$$

The coalescence rate constant, k_c, could be converted to a first order half life ($T_{\frac{1}{2}}$) for more convenient comparison of stability of different systems.

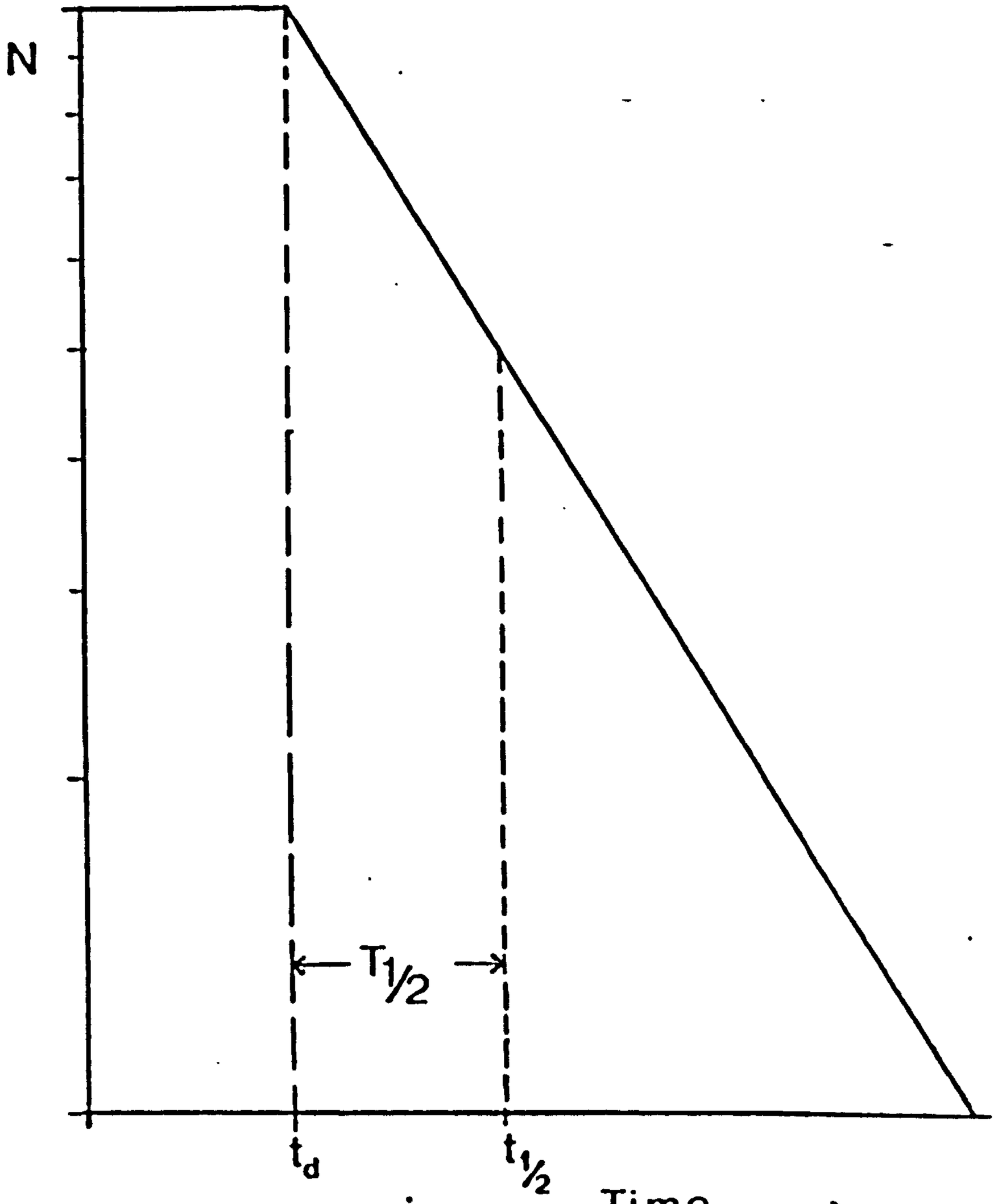
$$T_{\frac{1}{2}} = 0.693/k_c$$

The general form of such a plot is shown in Fig 4 . The stability could also be characterized by comparing the time required for half the droplets observed to coalesce ($t_{\frac{1}{2}}$), which may be read directly from the graph. The film drainage time (t_d) may be estimated from the graph or calculated using the following equation:

$$t_{\frac{1}{2}} - T_{\frac{1}{2}} = td$$

The mean rest-time (t mean) is another parameter which may be

Figure 4 Parameters for the Expression of Single Droplet Stability at the Plane oil/water Interface



N -- Number of droplets not coalesced

t_d -- film drainage time

$t_{\frac{1}{2}}$ -- time at which half the droplets coalesced

$T_{\frac{1}{2}}$ -- First order half-life for coalescence

calculated from these data (Jeffreys & Hawksley, 1962; and Edge & Greaves, 1967).

Additional, empirical expressions have been reported to correlate droplet stability data. Elton & Picknett (1957), correlated their experimental data for systems containing electrolyte using an equation of the form:

$$F_c = \alpha t^n/(1+\alpha t^n)$$

The constant, α, and n were found to be dependent on the electrolyte concentration, droplet size and temperature. F_c is the fraction of droplets which has coalesced in a given time, t.

A similar equation:

$$\log (N/N_0) = \alpha t^n$$

where N_0 is the total number of drops timed and N is the number of drops not coalesced after time, t, has also been used (Jeffreys & Hawksley, 1962; and Edge & Greaves, 1967).

Gillespie & Rideal (1956) found that for the single droplet rest times obtained in the absence of any added surfactant the following equation fitted the data:

$$\log (N/N_0 = -\alpha(t-t_0)^{3/2}$$

(where α is a coalescence constant and t_0 is the minimum rest-time). They derived this equation from theoretical considerations based on a model of a deformable drop resting at a plane interface. A number of workers have expressed reservations regarding the adequacy of this equation when applied to systems containing a surfactant. Nielsen et al (1958) found that better correlation was obtained by plotting $\log (N/N_0)$ Vs. t, and Jeffreys & Hawksley (1962) found it necessary

to vary the exponent. Edge & Greaves (1967) suggested that combinations of values of t_0 and the exponent were possible. However, other workers have reported a measure of agreement with this equation (Charles & Mason, 1960; Glass, Lundberg & Bailey, 1970), particularly for longer rest-times.

Alternatively, the coalescence data may be analysed by considering droplet rest-times about t mean. The simplest distribution is the normal distribution where the rest-times will be symmetrically distributed about the mean. Edge & Greaves (1967) have reported an approximate fitting of data, of first-stage coalescence times for the system of decanoic acid-heptane-water, by the normal distribution in the range 90% >N>10%. But, the normal distribution can give negative initial rest-times if some droplets persist for longer times than 2t mean and it predicts rest-times which are as far below the mean as others are above it. In these circumstances a skewed type distribution function such as the log-normal may be better, in this case the frequency of observation of zero rest-time is also zero. The validity of the normal or log-normal distribution functions can be tested by plotting the percentage of droplets not coalesced on a probability scale against time or log-time respectively. If the distribution function is applicable a linear graph should result from which two parameters can be utilized to uniquely define the given system. These parameters are the value of t at 50%, M, (which assumes the value $t_{\frac{1}{2}}$ = t mean for normal, and $t_{\frac{1}{2}}$ for log-normal distributions) and the scatter about M which is given the symbol, σ, (the standard deviation or geometric standard deviation for normal and log-normal distributions respectively).

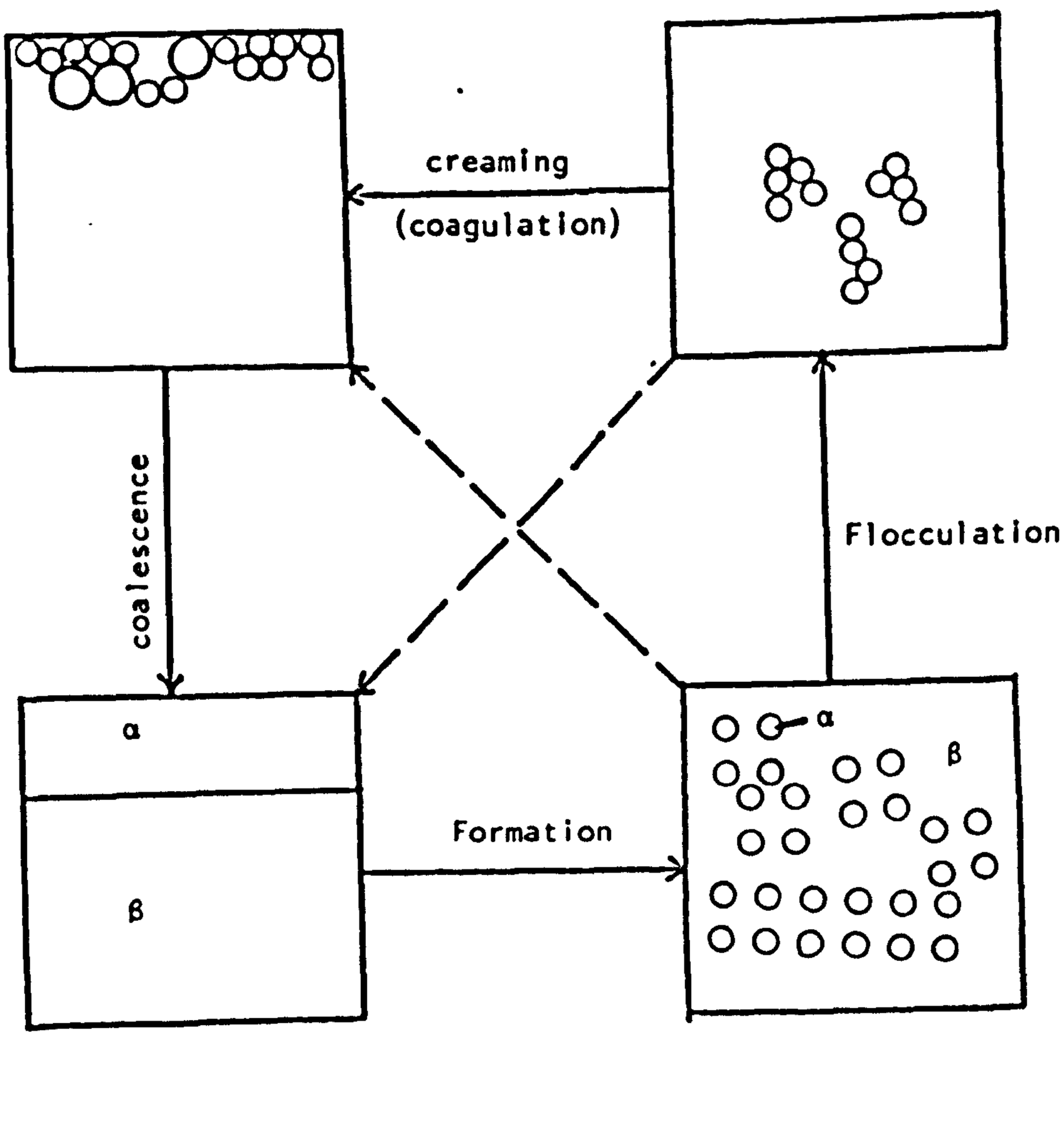
3.3 Bulk Stability

The problem of assessing emulsion stability is a controversial one. Accelerated stability tests are often employed such as centrifugation and storage at elevated or lowered temperatures, but in most cases these do not correlate well with the bulk stability of emulsions under normal storage conditions. Groves (1970) and Sherman (1971) have reviewed the various accelerated stability tests used for emulsions. These have already been discussed in subsection 1.2.2.5. The mechanisms by which instability results have been described in subsection 1.2.1 (also, see figures).

Accelerated ageing tests at either elevated or lowered temperatures may be useful provided the operative temperature does not go beyond the limits at which instabillity proceeds as at normal storage temperature. It has often been assumed that when emulsions are not too viscous, their stability under normal conditions can be predicted from their resistance to dispersed phase separation when subjected to high speed centrifugation (Merrill, 1953; Lotzkar & Maclay, 1943; Hayter, 1962). However, no serious attempt has been reported trying to correlate stability to centrifugation and stability during prolonged storage. Recently, Mittal (1975) has discussed in detail the ultracentrifugal technique in the study of emulsions. He concluded that "in spite of the difficulties and uncertainties associated with this technique, the method has numerous advantages over the conventional methods. The technique is very 'time saving".

Bulk stability of emulsions was measured by determination of particle size distributions directly, employing electron-microscopy. The

Figure 5 Schematic representation of the various stages in emulsion breakdown and formation. The dotted lines indicate that creaming can occur without flocculation and coalescence without coagulation.



changes in particle size distributions with time were determined. Emulsions were also subjected to elevated and lowered temperatures, and to centrifugal stress, in an attempt to correlate these results with bulk stability at room temperature. Particle size analysis was carried out of emulsions subjected to temperature stresses.

3.3.1 Preparation of emulsions

The emulsions were prepared using an ultrasonic probe (Dawe Soniprobe-7532A). The oil phase was placed in the 'rosette' flask (Fig 6) and the aqueous phase containing the surfactant was added. The tip of the sonic probe was placed at the interface and the mixture sonicated for 5 minutes at full power output. The 'rosette' flask was placed in an ice-bath during sonication. The design of this flask promotes cooling as well as mixing. The emulsions were stored in medicine bottles.

3.3.1.1 The Fluoride Ions

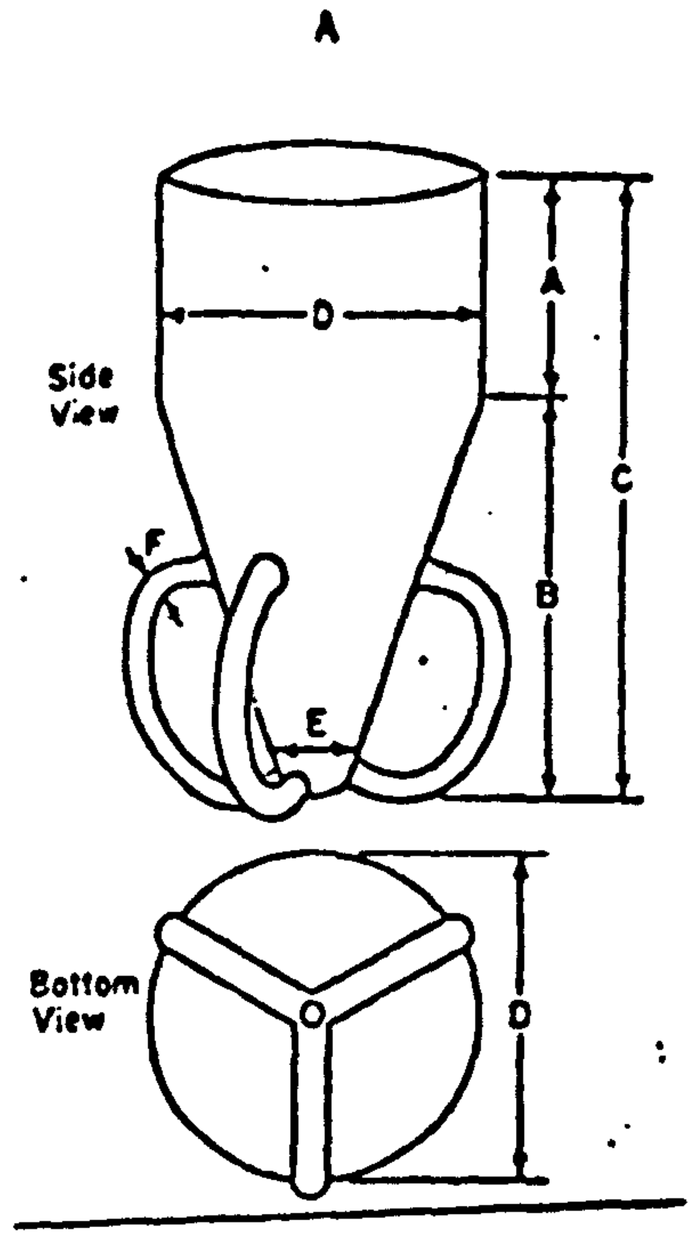
It has been reported that sonication can break the carbon-fluorine bond giving rise to fluoride ions (Clark, et al, 1975). This was investigated, using a calibrated fluoride selective electrode to measure the concentration of fluoride ions.

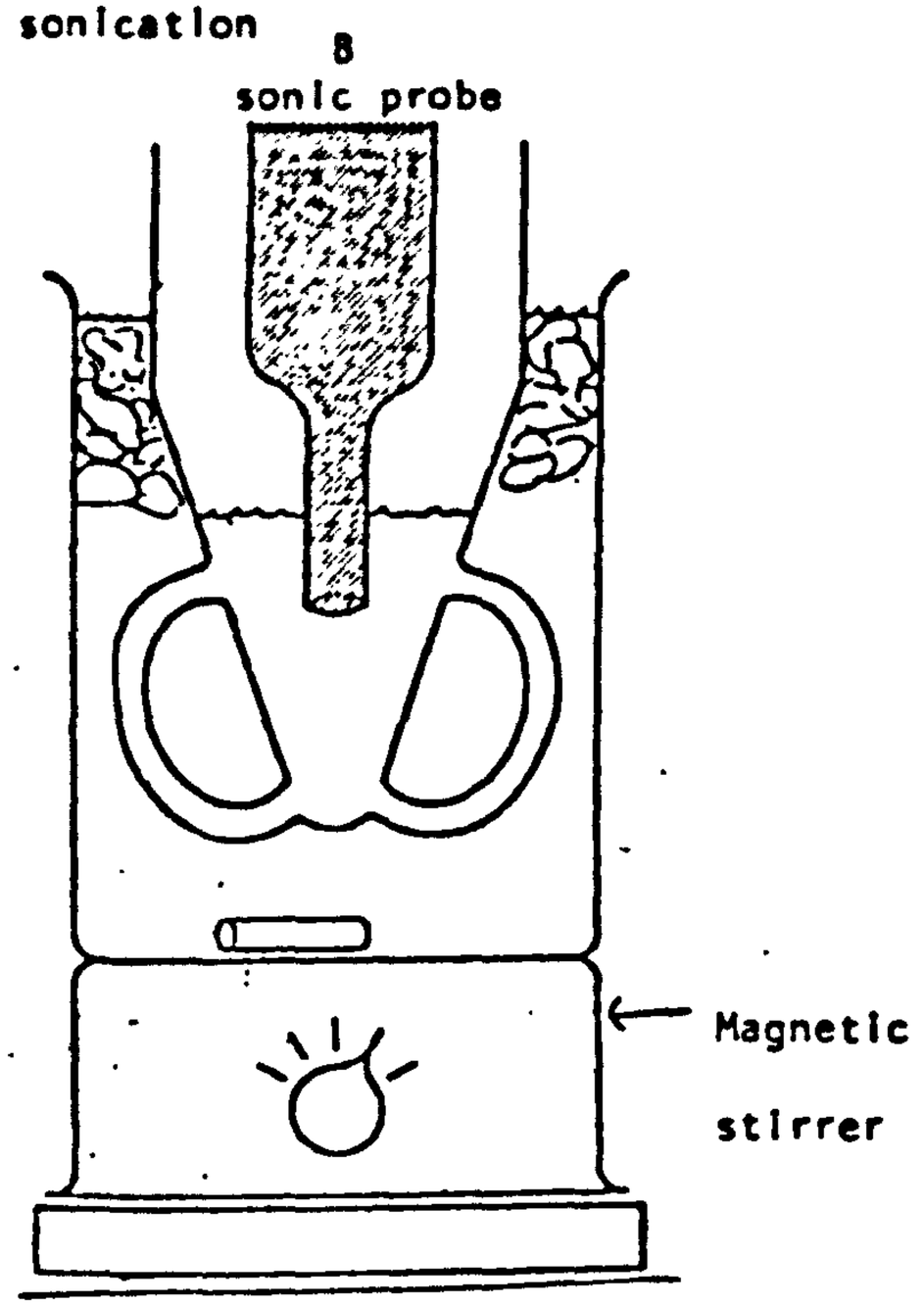
It is important to eliminate the fluoride ions from the emulsion which is to be infused since it can cause death if there is high enough concentration. It has also been found that the fluoride ion can act synergistically with the depressant effect of the anaesthesia and other factors which may lower cardiac output.

Figure 6

A. The Rosette Flask

B. Apparatus arrangement for Emulsion Preparation by sonication





3.3.1.2 Selective Fluoride-sensitive Electrode

The fluoride-sensitive electrode (Activion, type 003-15-008) could determine directly the concentration or activity of fluoride ions. The determinations could be carried out in aqueous or certain non-aqueous solutions or in suspensions.

3.3.1.2.1 Principle of Measurement

Electrochemically the electrode behaviour is similar to that of the glass electrode. The electrode potential (E) follows the Nernst equation down to a lower concentration limit of about 1×10^{-5} g ion per litre.

$$E = E_0 + \frac{RT}{zF} \ln A_{F^-}$$

where E_0 is a constant (volts)

R is the universal gas constant ($8.314 JK^{-1}mol^{-1}$)

T is the absolute temperature (^0K)

Z is the valency of the ion (in this case Z = -1)

F is the Faraday number (96487 As/g equ.)

A_{F^-} is the activity of the F^- ions (g ion/litre).

In a way similar to that used in connection with pH, let us introduce

$$pF = -\log A_{F^-}$$

Therefore we may write

$$E = E_0 + 2.303 \frac{RT}{F} pF$$

It is apparent from this equation that if E is plotted against pF, at a constant temperature, it will give a straight line. This linearity, in practice, ceases to exist at concentrations lower than 10^{-5} g ion per litre. However, the F^- ion activity can be determined down to a concentration of 5×10^{-7} g ion/l, using a previously

established calibration curve (Fig.7). The value of the factor $2.303 \frac{RT}{F}$ at different temperatures is given in table 3.3.1.2-1 (literature supplied for the electrode, Activion Ltd.).

Table 3.3.1.2-1 : (Activion Ltd.)

Temperature		$2.303 \frac{RT}{F}$
^{o}C	^{o}K	(mV)
10	283	56.1
15	288	57.1
20	293	58.1
25	298	59.1
30	303	60.1
35	308	61.1
40	313	62.1
45	318	63.1

3.3.1.2.2 Procedure

The EMF of the measuring cell was plotted against $-\log A_{F^-}$ for solutions of NaF of known concentration. The activity of these solutions was calculated knowing the activity coefficient (Activion Ltd.) see table 3.3.1.2-2. All measurements were carried out at 25°C. This graph was used as the calibration curve.

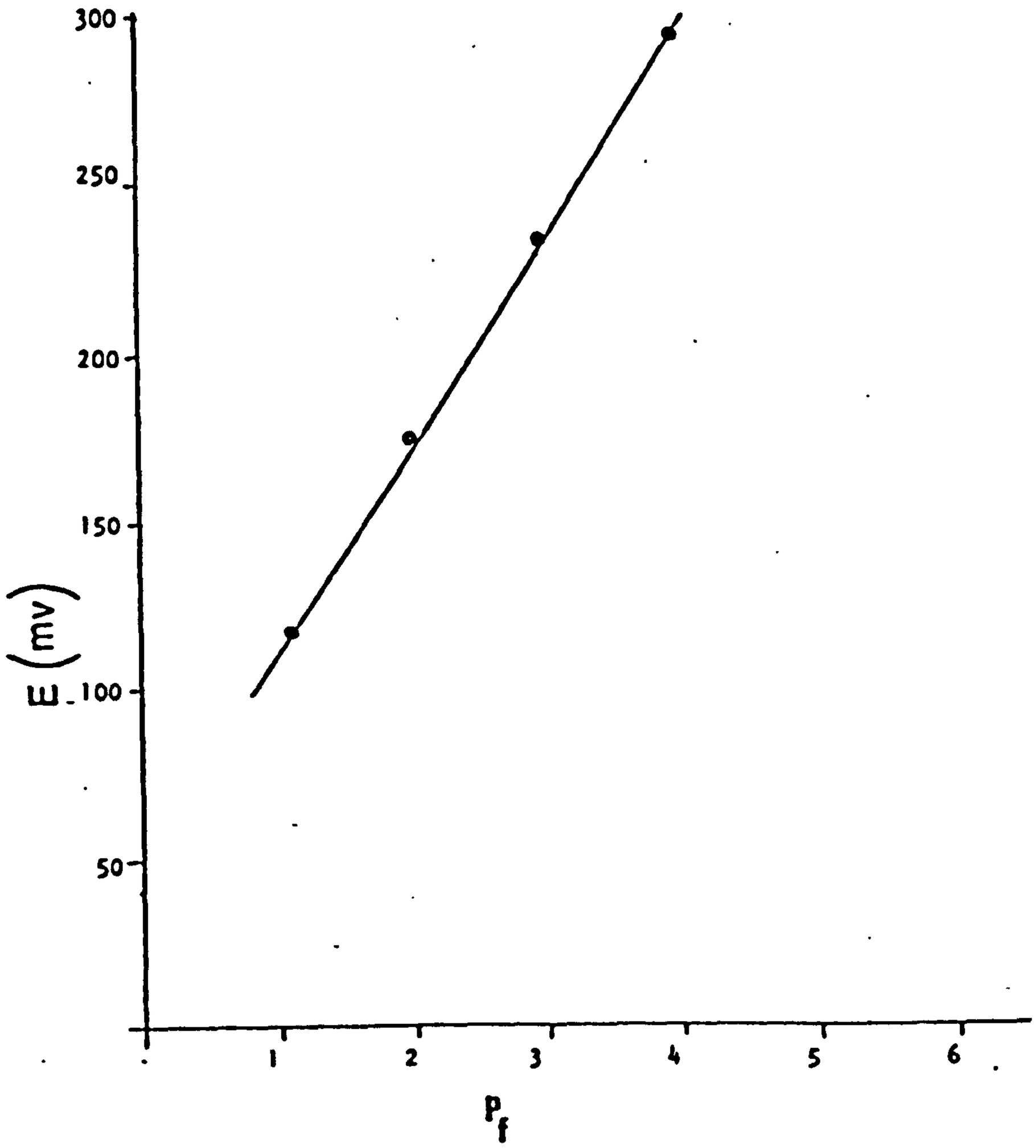
$$A^{F^-} = f^{\pm} C_{F^-}$$

where $f^{\pm}$ is the activity coefficient of the fluoride ions

A_{F^-} is the activity of fluoride ions.

C_{F^-} is the concentration of fluoride ions.

Figure 7 The Calibration graph used in the measurement of the fluoride ion concentration



The e.m.f. of the test solution was measured and the concentration of fluoride ions in it was obtained directly from the calibration graph.

Table 3.3.1.2-2 : Calibration Graph data

NaF Concentration (M)	Activity Coefficient	Activity (M)	pF	E.M.F. (E_o) (mV)
10^{0}	0.57	5.7×10^{-1}	0.219	-
10^{-1}	0.77	7.7×10^{-2}	1.114	52.9
10^{-2}	0.91	9.1×10^{-3}	2.048	55.2
10^{-3}	0.97	9.7×10^{-4}	3.015	57.2
10^{-4}	1.00	10^{-4}	4.000	58.2

3.3.1.2.3 Precautions in the handling of the Electrode

The following precautions were recommended by the manufacturers. The solutions used for measurements should be unbuffered with respect to the fluoride ions. When establishing the calibration curve, it is preferable to start from the more dilute solutions and in this case it is not necessary and even wrong to wash the electrodes between the measurements. On the other hand, the electrodes should be washed thoroughly with distilled water, if the e.m.f. is to be measured in a more dilute solution after having measured in a concentrated one.

Prior to placing into a new solution, both electrodes should be wiped dry with a piece of clean filter paper. The sensing element of the electrode should not be touched by hand, and it should not come into contact with any metal parts during storage. These precautions were strictly adhered to during all measurements. Prior to its first use, the electrode was preconditioned by letting it stand in a NaF solution of 10^{-1}M concentration for two hours.

3.3.2 Determination of Particle Size distribution

It has been reported that submicron size of droplets is desirable for fluorocarbon emulsions to be infused intravenously (Yokoyama et al 1974). A mean particle size of about 0.2 micron or less has been suggested as ideal. This small particle size poses problems regarding the measurement of droplet size distribution. One method based on sedimentation, using Stoke's law, has been reported (Fujita et al 1971). Electron microscopy, light scattering and low-angle X-ray scattering have also been suggested as possible methods for measuring particles of emulsions which are in the submicron size range (Paddy, 1969; Shinoda & Friberg, 1975).

All particle size distributions were measured by the use of electron microscopy and the Zeiss particle size analyser. This method was also compared with the sedimentation method.

3.3.2.1 Electronmicroscopy

Electronmicroscopy was used for direct examination of the emulsion droplets. Electronmicrographic image was produced on a photographic film by means of an electron beam, (A.E.I. Ltd., type: Corinth).

Since most materials are opaque to an electron beam even when only a few hundred angstroms thick, special problems exist in the production of suitably mounted specimens.

3.3.2.1.1 Specimen Preparation and Operation

Microscope-slide cover-slips were washed and thoroughly rinsed in distilled water, alcohol and dried. These cover-slips were coated with a thin film of formvar (Polyvinyl formal) by dipping in a 1% solution of formvar in Chloroform. A few drops of the emulsion were placed on a coated cover-slip and thinly spread over it, removing the excess emulsion. The cover-slips were placed in covered petri-dishes to avoid dust and allowed to dry at room temperature. The cover-slips with the specimen were coated with a carbon film under vacuum by electrical discharge (low tension, 60 amps) from two pointed hard graphite rods (N.G.N. Coating Unit, model 12 SG-2).

The cover-slips were removed from the coating unit and the carbon film cut into small squares (4 mm approximately) using a razor blade. The cover-slips were then immersed in chloroform to dissolve away the formvar film leaving the carbon replica floating. The small squares of the replica are rolled up at this stage but are unrolled in water. Since water and chloroform are relatively immiscible, the replicas were first rinsed in alcohol before floating them onto water. Copper grids were used to transfer the replicas from one solvent to another. The carbon replica squares were finally mounted on copper grids 3 mm in diameter and 100 mesh, allowed to dry in petri-dishes and shadowed (for good contrast) with a 40% palladium-gold alloy wire in the same coating unit under vacuum by electrical evaporation of the alloy wipe. The grids were now ready to be mounted in the electron microscope.

Electronmicrographs of emulsion particles were taken at suitable magnification and from many different squares of the grid in order to get a representative sample. Furthermore, there were four such grids prepared for each emulsion.

The use of plastic petri-dishes to store the grids was avoided since the static electricity on plastic causes the grids to stick to it thus destroying the replica. The magnification of an electronmicroscope can vary as much as 8% through normal use especially at high magnifications. Therefore the microscope was calibrated regularly and as an additional precaution a picture of latex particles of known size range was taken every time the microscope was used.

3.3.2.1.2 The Zeiss Particle Size Analyser

The Zeiss particle size analyser was used to measure particle size distributions directly from the electronmicrographs (Carl Zeiss Ltd., West Germany, model TGZ 3).

The measuring procedure consisted of reading the summation counter (which cannot be set to zero) and then zeroing the individual counters by depressing the red buttons. The electronmicrograph was placed on the desk and the brightness of the measuring mark and of the surrounding field adjusted so that the measuring mark and the surrounding field were clearly recognizable. The photograph was moved by hand on the desk until the centre of the particle was at the centre of the measuring mark and the foot switch depressed. This released the counting procedure and the particle was perforated by the marking pin. By this method 1000 to 3000 particles were sized for each emulsion.

3.3.2.2 Centrifugal sedimentation Vs. Electronmicroscopy, as methods for the determination of particle size distribution of fluorocarbon emulsions.

Recently, Yokoyama and Coworkers (1974) have proposed a sedimentation method for specifying the particle size distribution based on Bostock's theory and Stoke's law. This method was compared with electronmicroscopy. The theory involved in the sedimentation method is as follows:-

Let us assume that the quantity of perfluorinated compound (ΔW) in the emulsion particles with a diameter, D, distributes in compliance with equation 1.

$$\Delta W = F(D) \text{ ----------Eq. 1.}$$

where F is a function.

Then the substance fraction (Wt) in emulsion particles larger than the diameter Dt is given by:-

$$Wt = \int_{Dt}^{D\infty} F(D)dD \text{ ----------Eq. 2.}$$

See diagram 1.

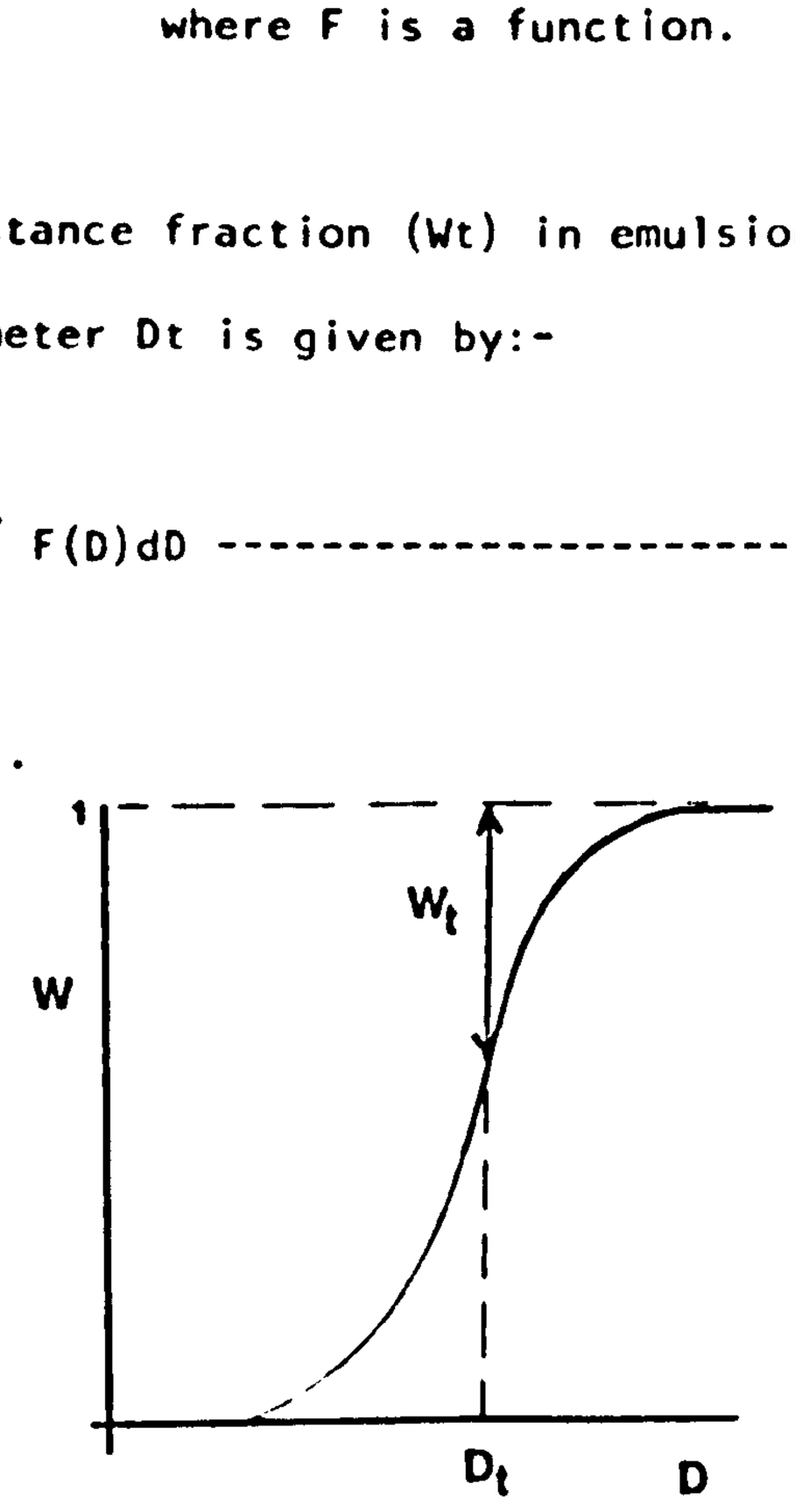


Diagram 1. Wt and Dt.

According to Bostock's theory, the weight per cent (P) which has settled out of a certain upper portion in the fluid column of a centrifugation tube, at time, t, consists of two parts:-

$$P = \int_{Dt}^{D\infty} F(D)dD + \int (vt/x)F(D)dD \quad \text{---------- Eq. 3.}$$

The first term on the right-hand side of equation 3 consists of all the particles with a larger diameter than Dt which is given by equation 4 applying Stoke's law.

$$Dt = \sqrt{\frac{18\eta . \ln[(M+x)/M]}{(\rho e-\rho m)\omega^2 t}} \quad \text{---------- Eq. 4.}$$

Where M is the distance between the surface of the emulsion in the centrifuge tube and the axis of the centrifuge, and

x is depth from the surface to the bottom of the portion in the fluid column,

ω is speed of rotation of centrifuge,

η is the coefficient of viscosity of the medium,

ρe and ρm are densities of particle and medium respectively.

The second term on the right-hand side of equation 3 consists of particles smaller than Dt which are sedimented out of the portion starting from some intermediate position in the fluid column. If the mean velocity of sedimentation of these smaller particles is v, the fraction of particles of this size that is driven out of the portion is vt/x.

Differentiating equation 3 with respect to t and multiplying by t

gives:-

$$t\frac{dP}{dt} = \int_{Dmin.}^{Dt} (vt/x)F(D)dD \quad \text{---------- Eq. 5.}$$

Substituting into equation 3:

$$\text{From Eq. 2 ------ } Wt = \int_{Dt}^{D\infty} F(D)dD$$

$$\text{and " Eq. 5 ------ } t(dP/dt) = \int_{Dmin.}^{Dt} (vt/x)F(D)dD$$

gives:

$$P = Wt + t(dP/dt) \quad \text{---------- Eq. 6.}$$

$$\text{Therefore } Wt = P - \frac{dP}{d(ln.t)} \quad \text{---------- Eq. 7.}$$

Thus if P and t are known it is possible to determine Wt by using equation 7.

Method:-

Two emulsions, A and B, were prepared using the ultrasonic probe. Emulsion A contained perfluorotributylamine 20% w/v and Pluronic F-68 4% w/v as the emulsifier. Emulsion B contained perfluorotributylamine 20% w/v emulsified with lecithin and Pluronic F-68 3% w/v of each. The particle size distribution of each emulsion was determined by electronmicroscopy as described in 3.3.2.1 and by the centrifugation method as follows.

A 10 ml sample from each emulsion was poured into a centrifuge tube. The tubes were then placed in a swing type rotor and immediately centrifuged. The distance from the centre of rotation to the surface of emulsion was adjusted to be 10 cm. After centrifugation, the upper 2 cm. portion from the surface was carefully removed with a syringe and analysed for quantitative determination of perfluorotributylamine (FC-43), content using Gas Chromatography as described in subsection 3.3.2.2.1. Dt was calculated from equation 4 for the various centrifugal conditions.

3.3.2.2.1 Gas Liquid Chromatography

Gas chromatography was used for quantitative analysis of the fluorocarbon oils. In the past many workers used the oxygen flask combustion method to quantitatively determine the fluorinated compounds. But this method was not applicable to the perfluorocarbon oils investigated because the CF_3 groups in them could not be completely decomposed by the "oxygen method" thus giving unsatisfactory quantitative analysis. Recently, Yokoyama and Coworkers (1974) have described gas chromatographic technique for determining fluorocarbons quantitatively.

The gas chromatograph used in our investigations was Perkin-Elmer model F-17, equipped with hydrogen flame ionization detector. A glass column, ¼ inch outer diameter and 2 metres long, packed with 20% silicone OV-17 on chromosorb W AW, 60-80 mesh, was used (Perkin-Elmer Ltd.). The column was carefully conditioned prior to use according to makers recommendations as follows:
The detector end was disconnected and the nitrogen flow rate set at approximately 10 ml/min. After purging at room temperature the

column oven was slowly (1°C/min.) raised to the required temperature. The column was kept at this temperature until no signal could be detected i.e. there was no more material bleeding from the column.

The operation conditions were as follows:-

Column oven temperature 60°C, injection port temperature 175°C, nitrogen gas flow rate 40 ml per min; air and hydrogen inlet pressures (after ignition of detector) 15 lb./sq. in. each. Benzotrifluoride (BTF) was used as the internal standard and 1,1,1-trichlorotrifluoroethane (FC-113) as the solvent. Standard solutions were prepared in FC-113 each containing an accurately known volume ratio of a given fluorocarbon oil and BTF. These standard solutions were used to prepare calibration curves for each fluorocarbon oil (FC) investigated. The calibration curves were obtained by plotting peak height ratio (FC/BTF) Vs. Volume ratio (FC/BTF) x-axis. An average of three readings was taken for each point on the graph. Figures 29 and 30.

3.3.3 Viscosity Measurement

A standard U-tube viscometer (model A, Griffin and George Ltd.) was used to measure the viscosities of the emulsions. This viscometer gave a flow time of 316 $\pm$ 0.5 seconds for triple distilled water at the operating temperature of 25°C $\pm$ 0.05°C (Townson and Mercer Ltd., series III bath, with viscometer type lid). The U-tube was cleaned with chromic acid and rinsed thoroughly with distilled water, twice with acetone and dried in hot air. 30 minutes were allowed for thermal equilibrium of the viscometer and emulsion before filling the viscometer. One flow time was measured immediately and twice again to get a mean flow time. The viscometer

Figure 29 Showing typical GLC peaks obtained in quantitative fluorocarbon chemical analysis.

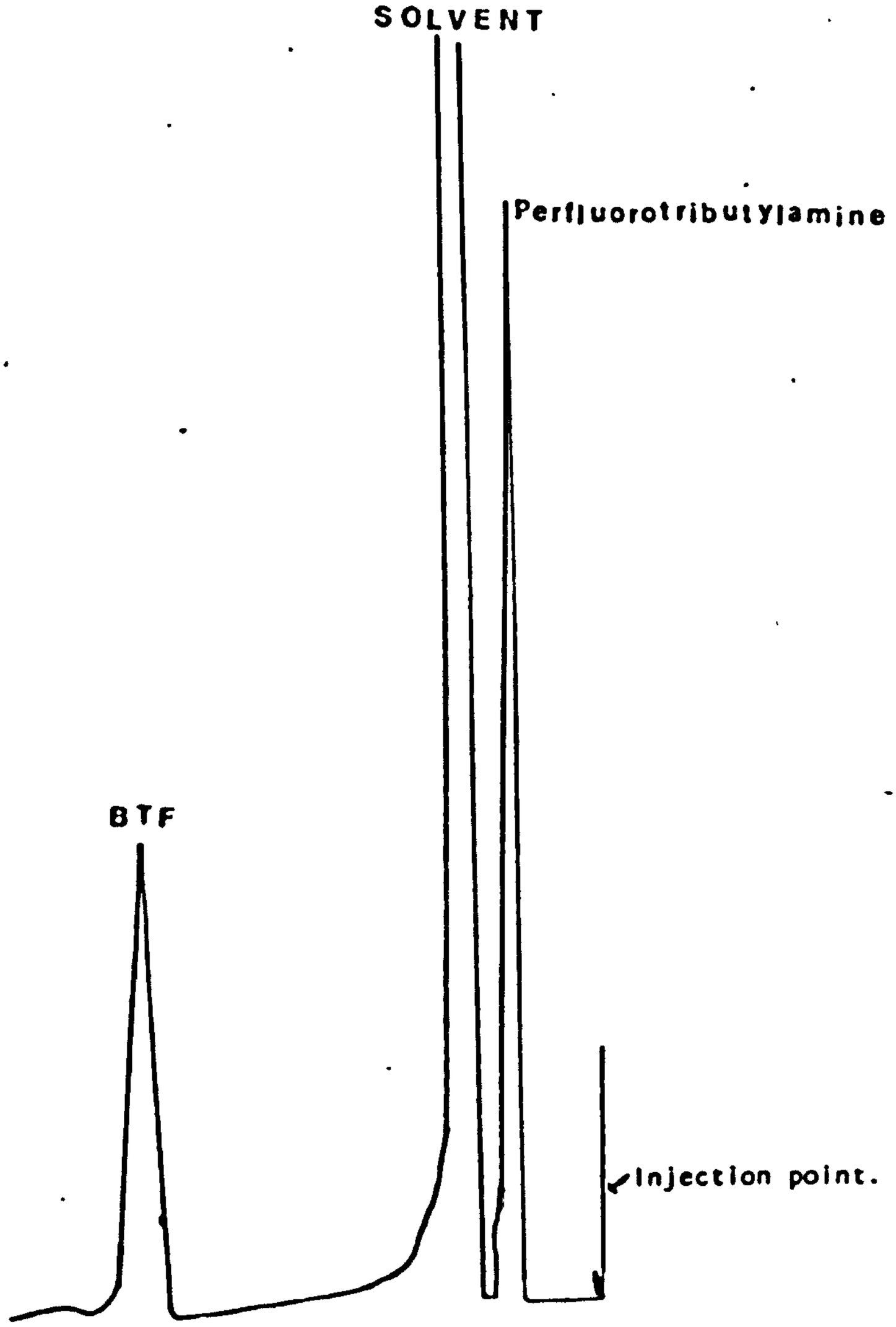
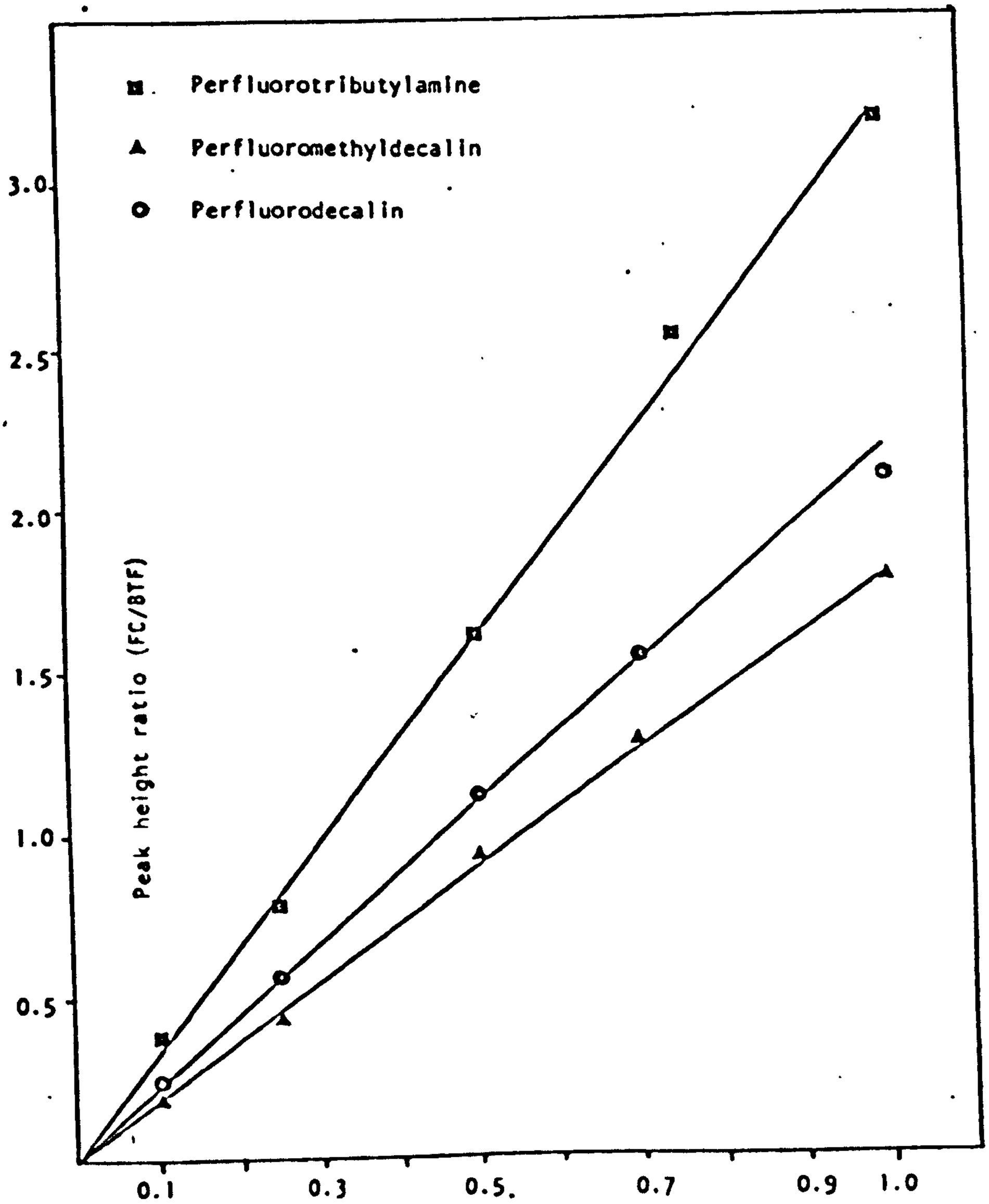


Figure 30 Showing Calibration graphs used in quantitative analysis of fluorocarbons by G.L.C.



was emptied and cleaned between each different emulsion. The relative viscosities of pure liquids were also measured by this method.

The following equation was used to calculate the relative viscosity

$(\eta_r = \eta_1/\eta_2 = \rho_1 t_1/\rho_2 t_2$

where t_1 and t_2 are the mean flow times of the two liquids of densities ρ_1 and ρ_2 respectively.

3.3.4 Sterilisation of Emulsions

A sterile preparation is one that is free from living micro-organisms. In addition, it must not contain viable bacterial spores, yeasts and mould spores. Two methods were used to sterilise the fluorocarbon emulsions, autoclaving and filtration.

3.3.4.1 Sterilisation by Steam

A portable standard laboratory steriliser was used. The emulsions to be sterilised were placed in glass containers, stoppered by rubber bung which was retained in position by a crimped metal ring. The emulsions were autoclaved at a pressure of 20 pounds per square inch (equivalent to 126^{o}C, Cooper & Gunn, 1970), for 15 minutes. Sterility tests and particle size analysis were carried out on the autoclaved emulsions.

3.3.4.2 Sterilisation by Filtration

The filtration was carried out in a cabinet which had a continuous flow of sterile air (Hepaire Ltd.). The emulsion to be sterilised was placed in a 50 ml syringe and forced through a millipore

filter having 0.45 μ pore size. The main disadvantage of this method was that it was cumbersome and a certain amount of the product was lost during the process. Sterility tests and particle size analysis were carried out on the filtered emulsions.

3.3.4.3 Sterilisation by Steam Combined with Filtration

The idea behind combining the two methods was to see if by prior filtration the emulsions could be sterilised at a lower steam pressure, which means lower temperature, since higher temperature may adversely affect the emulsions regarding stability. Two variations were investigated and each time sterility tests and particle size analysis were carried out.

(A) The oil phase and the aqueous phase containing the emulsifier were filtered, under aseptic conditions (sterile air cabinet), through a millipore filter having 0.45 micron pore size. Then the emulsion was prepared in an enclosed atmosphere (the sonic probe in a cabinet the inside of which had been wiped with chlorhexidine solution) using sterile apparatus and autoclaved sonic-probe tip. The resulting emulsion was placed in glass container and autoclaved at 10 pounds per square inch steam pressure for 15 minutes.

(B) The emulsion was prepared, filtered in open atmosphere through a millipore filter having 0.45 micron pore size and the resultant emulsion autoclaved at a pressure of 10 pounds per square inch for 15 minutes.

3.3.4.4 Sterility Tests

Sterility tests were carried out for the detection of aerobic and anaerobic micro-organisms. For the detection of aerobes peptone broth and for anaerobes thyoglycolate medium were used. A one ml sample from the emulsion under test was transferred to a bottle containing 40 ml of the appropriate growth medium (large volume of growth medium used to ensure that any growth inhibiting action of surfactants was diluted out). Three such replicas were made, one incubated at 35 to 37°C, one at room temperature and the third at 4°C in the refrigerator. At each temperature a control was also incubated. Thus there were 12 bottles altogether for each emulsion tested, 6 were sample bottles (3 for aerobes and 3 for anaerobes) and 6 control bottles (3 for aerobes + 3 for anaerobes). The incubation at room temperature and at 4°C was an attempt to simulate storage conditions at these temperatures. Incubation time in all cases was 7 days, but samples were examined periodically for any growth of micro-organisms.

The controls contained the appropriate micro-organisms in the appropriate growth medium (aerobe or anaerobe) and one ml of emulsion. The emulsion was included to demonstrate that the medium was capable of supporting growth of micro-organisms in the presence of the sample.

3.3.5 Storage

The effect of storage at various temperatures on the stability of fluorocarbon emulsions was investigated. The effect of freeze-thaw cycles and the container was also investigated.

3.3.5.1 The Effect of Temperature

The emulsions under investigation were stored at 50°C in an oven, room temperature (18 - 25°C), in the refrigerator (4 - 6°C) and in the deep-freeze (-4 to -10°C). At suitable time intervals particle size analysis was carried out on each emulsion. In addition the effect of freeze thaw cycles on the particle size distribution of emulsions was investigated.

3.3.5.2 The Effect of the Container

Emulsions were stored in clear glass containers, amber coloured glass containers and plastic containers and stored at room temperature. Particle size analysis was carried out at suitable time intervals to see the effect on stability of the emulsion.

3.3.5.3 General Observations

All fluorocarbon emulsions showed sedimentation of the oil droplets on storage. The sediment volume was measured after storage for a pre-set time interval. The sediment could be easily redispersed by shaking the container. The emulsions prepared with egg-lecithin as the emulsifier, which were non-sterile, showed considerable growth of micro-organisms after storage for two months at room temperature.

3.3.6 The Effect of the Nature of the Oil Phase

It has been reported (Davis & Smith, 1975) that the nature of the oil phase plays an important role in the stability of the emulsion. Experiments were carried out to see if this holds true in case of the fluorocarbon oils. Emulsions were prepared containing different fluorocarbon oils but the same emulsifier, and emulsions which

contained, in the oil phase, small concentrations of a given additive to evaluate its effect on stability. The emulsions were stored at room temperature in medicine bottles and particle size analysis was carried out at suitable time intervals. Similarly various different emulsifiers were investigated as well as emulsifier systems (two or more emulsifiers combined).

3.3.7 Forced Coalescence

It has been reported (Mittal, 1975; Cocktain, 1952) that forced coalescence of emulsion droplets in a centrifuge may be a useful accelerated stability test to predict the bulk stability of emulsions on storage. This method was evaluated to see if it could be used as an accelerated stability test.

The emulsions were prepared and placed in 25 ml plastic centrifuge tubes with brass stoppers incorporating '0' ring seals (MSE Ltd.). The tubes were placed in a high speed rotor (fixed angle) and the emulsions were centrifuged at 25,000 r.p.m. (equivalent to 74,000 g) for one hour (MSE Ltd. High Speed Centrifuge, Model 25). After centrifugation the tubes were removed and the volume of oil phase which had separated was measured.

3.4 Miscellaneous Experiments

3.4.1 Density Measurement

All density measurements were carried out using a specific gravity bottle of 50 ml volume. First the density bottle was weighed empty, with stopper, then it was filled with distilled water, stoppered and allowed to thermally equilibrate to 25°C in a water bath (Townsend & Mercer Ltd.). The excess liquid escaping through the

hole in the stopper was carefully removed, the density bottle removed from the water bath using a pair of tongs and wiped dry with a tissue paper. Throughout the procedure care was taken not to handle the bottle since body heat could heat up the liquid inside the bottle and make it escape from the bottle giving erroneous results. The density bottle was weighed. It was repeated twice to get a mean reading for the weight of bottle filled with water. The whole operation was repeated using the test-liquid in the density bottle. The specific gravity (S.G.) was calculated by substitution into the equation below:

$$\text{S.G.} = \frac{\text{weight of liquid}}{\text{weight of water}}$$

The densities of the oil phases have been entered in table 2.3-2.

3.4.2 Boiling Point Determination

The boiling point of purified fluorocarbon chemicals was determined by simple distillation. All glass quick-fit apparatus was used. A distilling-flask (100 ml) was fitted with a stopper carrying an accurately calibrated thermometer, the bulk of which was just level with the side arm. The flask was connected, via the side arm, to a water cooled condenser. A flat-bottomed flask served as a receiver. The flask was heated directly with a slighly luminous bunsen flame. The temperature at which the liquid distilled was noted, this was the boiling point of the liquid. A mean of three readings was taken and entered into table 2.3-2.

3.4.3 Oxygen Solubility in the Fluorocarbon Emulsions

An oxygen electrode (Rank Brothers) was used to investigate the solubility of oxygen in fluorocarbon emulsions. This electrode

is specially designed for following the uptake or liberation of oxygen by suspensions.

3.4.3.1 Principle of Operation

Oxygen diffuses through a thin (0.0005 inch) teflon membrane and is reduced at a platinum surface immediately in contact with the membrane.

$$O_2 + 2E + 2H + \longrightarrow H_2O_2$$

$$H_2O_2 + 2E + 2H + \longrightarrow 2H_2O$$

where E stands for electron.

The other half-cell is also incorporated in the base of the incubation vessel and is composed of a Ag-AgCl electrode.

3.4.3.2 Setting up the Electrode

The base of the incubation vessel was detached by unscrewing the perspex locking-nut. Sufficient saturated potassium chloride solution was added to wet the silver and platinum electrodes. A piece of lens tissue one sq. cm. was cut and a 1 mm diameter hole was made in it. The tissue was placed over the platinum electrode so that the hole was over the electrode. A 2 cm. square piece of teflon membrane was cut and placed over the lens tissue and locked in position by putting the incubation vessel in place and screwing down the locking nut. Care was taken to avoid trapping air bubbles or twisting the membrane. The oxygen electrode was now ready for operation and was placed on the magnetic stirrer. A potentiometric recorder (MSD Ltd; Electro-plus) was used to record the percentage of dissolved oxygen.

3.4.3.3 Operation

The Ag-AgCl electrode was connected to the positive side of the potential divider of the recorder and the platinum electrode to the negative. Air saturated water was added to the incubation vessel and the perspex disc placed in position. After ensuring that no air bubbles were trapped the magnetic stirrer was switched on. The sensitivity control of the recorder was adjusted to give a minimum deflection. The whole cell was allowed to attain thermal equilibrium to 25^{o}C by circulating water through the incubation vessel.

The water in the incubation vessel was replaced by a fluorocarbon emulsion which had been saturated, in an all oxygen atmosphere, by bubbling oxygen through it. The reading on the recorder was adjusted to be at maximum. After steady state conditions had been reached the rate of release and take-up of oxygen from the emulsion was followed.

3.4.4 Phagocytosis of Fluorocarbon Emulsions

It has been reported that Phagocytosis by the reticuloendothelial system is the main mechanism by which fluorocarbon emulsions are cleared from the blood stream in-vivo (Yokoyama et al, 1975). Phagocytosis experiments were carried out in-vitro to investigate some of the factors which may affect the rate of phagocytosis.

3.4.4.1 Preparation of Buffer Solution

Hank's Balanced Salt Solution was used to sustain the activity of phagocytes. A twin-pack (Oxoid Ltd. Code BR 19A) was purchased which had Hank's solution in the dehydrated state. From this, sterile stock solutions 'A' and 'B' were prepared which keep

indefinitely without precipitation. The contents of pack 'A' were dissolved in one litre of deionised water and the solution sterilised by autoclaving at 121°C for 15 minutes to give stock solution 'A'. Similarly stock solution 'B' was prepared from pack 'B'. The use of two stock solutions avoids the precipitation which occurs when a concentrate of the complete solution is heated. The final culture media were prepared by adding 100 ml of solution 'A' and 100 ml solution 'B' to 800 ml deionised water. It was mixed and sterilised by autoclaving at 121°C for 15 minutes, allowed to cool to room temperature and aseptically 25 ml of buffered bicarbonate solution was added (Oxoid Ltd., Code BR 26).

Hank's solution serves to maintain pH and osmotic pressure and provides an adequate concentration of essential inorganic ions; (Davies et al, 1975). The formula of Hank's stock solution is given in table 3.4.4-1. The buffered bicarbonate solution was a sterile 1.4% w/v solution of sodium bicarbonate which had been buffered to pH 6.9 to 7.0 with carbon dioxide gas. After the addition of this solution to Hank's balanced salt solution the pH was adjusted to between 7.2 to 7.4 by loosening the cap of the bottle and allowing carbon dioxide to escape.

Table 3.4.4-1 : Hank's Stock Solution Formula

Part 'A'	Grams/litre
Potassium Chloride	0.4
Sodium Phosphate	0.06
Potassium dihydrogen Phosphate	0.06
Sodium Chloride	4.358
Phenol red	q.s.
Part 'B'	
Calcium Chloride	0.14
Magnesium Sulphate	0.1
Magnesium Chloride	0.1
Dextrose	1.0
Sodium Chloride	3.642

3.4.4.2 Preparation of Phagocytes

The method used for obtaining polymorphonuclear blood cells was based on that reported by W. Davies and Co-workers (1975). Guinea-pig blood was collected in 10 ml heparinized (10 units/ml) tubes. Buffer coat cells, obtained by gravity sedimentation, were centrifuged for 10 minutes at 100 g. The cell pellet was washed by resuspension and similar centrifugation in cold EDTA - saline (0.2% w/v EDTA, 0.85% w/v Nacl, pH 7.4). This buffer prevents clumping of cells by removing divalent cations. The cell pellet was finally resuspended in Hank's balanced salt solution at a concentration of 4 mg of cell protein per ml.

3.4.4.3 Assay of Phagocytosis

The Phagocytosis of emulsion particles was assessed by a turbidometric method. First a calibration graph was prepared relating the turbidity of the culture media to the number of particles in suspension.

After preliminary experiments had established the time course of particle uptake, the following standard incubation system was utilized. Cells (4 mg of cell protein per ml) were incubated in Hank's balanced salt solution, pH 7.4, total volume 10 ml, for 10 minutes in 25 ml glass bottles, at 37°C, in a shaking water bath set at 100 strokes per minute. Then a sufficient volume of freshly prepared fluorocarbon emulsion, prewarmed to 37°C was added to the flask so that the final particle concentration was 10^9 particles per ml. A duplicate was prepared to get a mean value of turbidity. The reference solution used in the measurements of turbidity was Hank's solution with cells only. A control was also used which contained everything except the cells.

Samples were taken at suitable time intervals, cooled to room temperature and the turbidity measured. If necessary (that is when absorption was outside the recorder range) the sample was diluted with Hank's solution. The turbidity was measured using a Beckman UV/visible spectrophotometer, model 25 in conjunction with a chart recorder (also Beckmann). The effect on the rate of phagocytosis, of particle size and charge was investigated.

4. RESULTS

4.1 Surface and Interfacial tension

4.1.1 Surface tension of surfactant solutions

The surface tensions of solutions of surface active agents, measured by the Wilhelmy plate method are tabulated in table 4.1-1. For each surfactant investigated, the log concentration versus surface tension was plotted and, from the inflexion point, the critical micelle concentration (c.m.c.) estimated. The c.m.c. values thus obtained were entered in table 2.2-2. Typical log concentration vs. surface tension graphs are shown in figure 8 The surface tension of the purified oil phases was also measured and listed in table 2.3-2.

4.1.2 Interfacial tension and adsorption

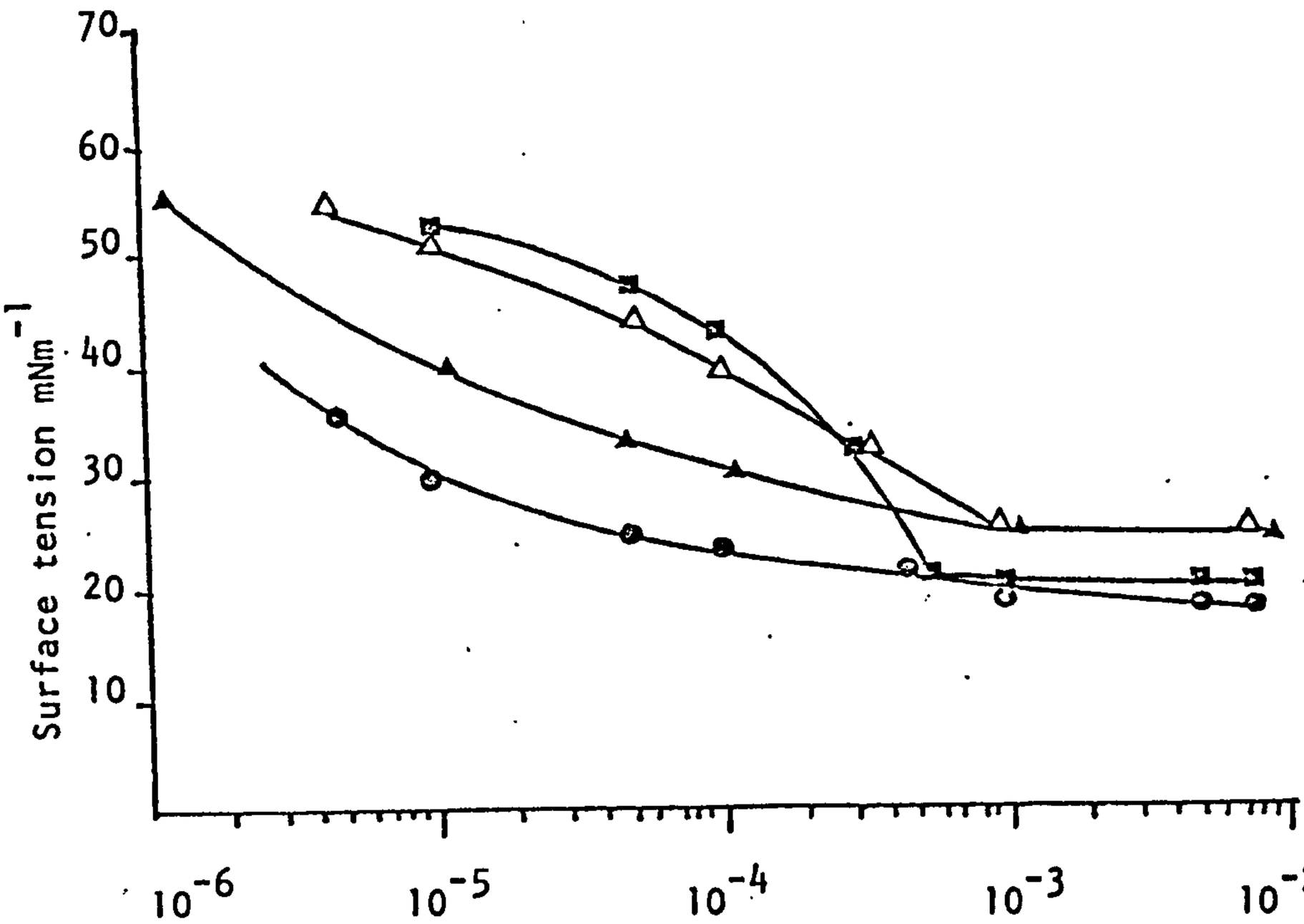
The interfacial tensions for a number of oil phases against water and solutions of surfactants were determined by the pendant drop method and are listed in table 4.1-2.

Interfacial tension vs. log concentration was plotted for each system investigated, typical graphs are shown in figure 9

The interfacial tensions show a clear dependence on the nature of the oil phase in the absence or presence of a surfactant.

A number of systems were investigated in more detail, measurements of interfacial tension (γ_{OW}) being recorded at a wide range of

Figure 8 Surface tension of some aqueous surfactant solutions



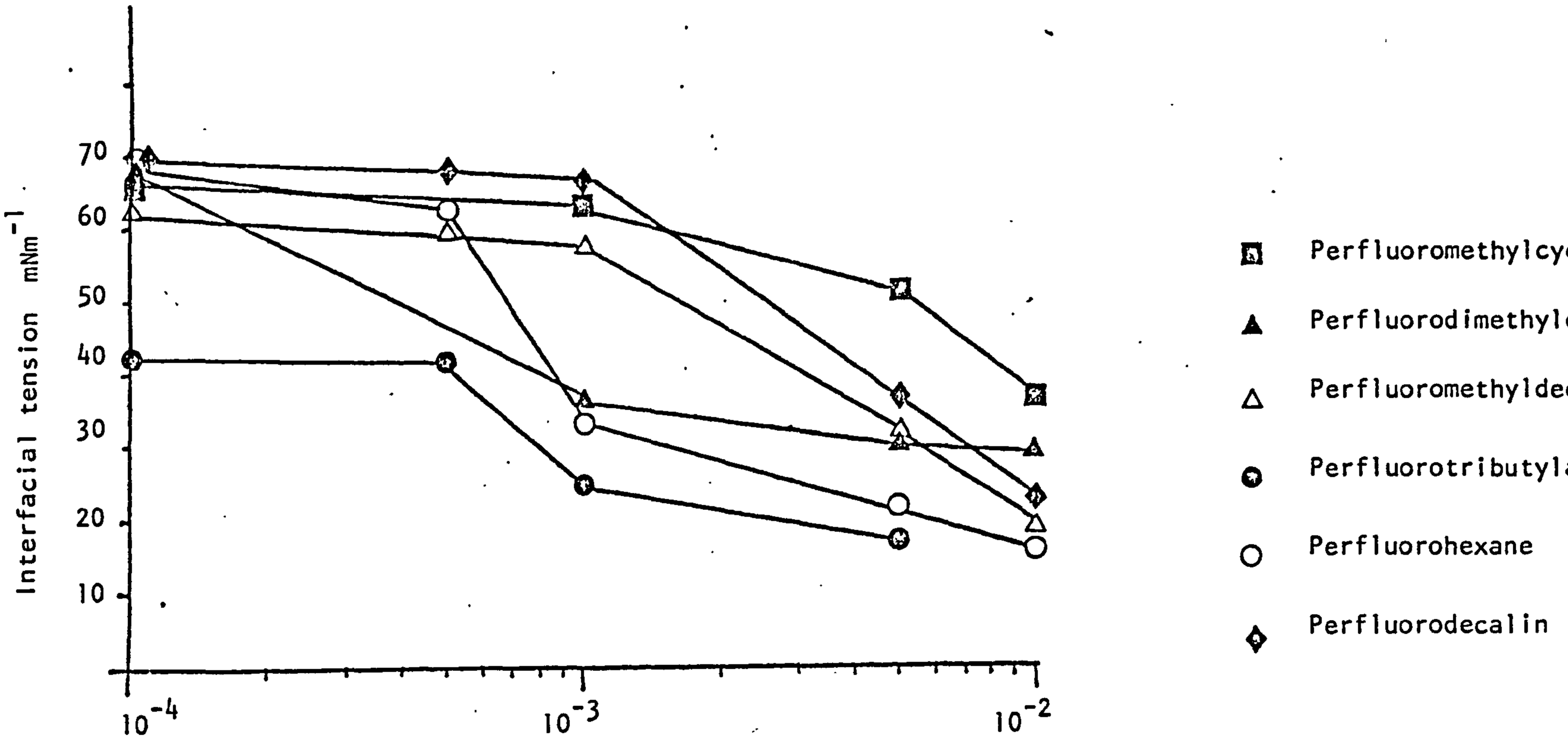


Figure 9 Typical Interfacial tension graphs

surfactant concentrations. From these data the Gibbs surface excess (see subsection 3.1.4, and appendix 2) of adsorbed surfactant and area per adsorbed molecule were calculated and listed in table 4.1-2.

Table 4.1-1 Surface Tension of Surfactant Solutions

Molarity of Aqueous solution of Surfactant	Surface Tension at 25°C mNm^{-1}								Molarity of SDDS Aqueous	Surface Tension at 25°C mNm^{-1}
	Forafac 1023	Forafac 1111	Forafac 1032	F.C. 170	F.C. 126	Monflor 51	Monflor 52	Pluronic F-68		
10^{-6}	-	-	-	52.1	60.2	55.5	52.5	53.27	10^{-4}	69.9
5×10^{-6}	-	35.2	55.5	-	40.6	-	40.0	51.30	5×10^{-4}	63.3
10^{-5}	52.1	30.0	52.0	35.2	30.0	40.0	34.1	48.37	7.5×10^{-4}	59.5
5×10^{-5}	47.5	26.3	45.1	28.3	20.8	31.4	-	47.4	10^{-3}	55.5
10^{-4}	42.5	25.0	40.0	24.2	20.0	30.0	27.2	46.44	2.5×10^{-3}	44.0
5×10^{-4}	21.8	22.5	34.1	20.5	17.5	-	26.0	44.6	5×10^{-3}	33.9
10^{-3}	20.5	20.1	27.6	19.5	15.0	26.9	28.0	43.8	6×10^{-3}	33.2
5×10^{-3}	20.5	20.0	-	-	-	-	-	43.0	7.5×10^{-3}	33.3
7.5×10^{-3}	20.5	20.0	27.0	19.0	14.6	25.0	23.4	-	10^{-2}	33.0

Table 4.1-2 Interfacial tension of Perfluorochemicals

Molarity of Surfactant Solution	Interfacial tension at 25°C (mNm^{-1})	Surface excess, Γ moles/cm^2	Area per molecule nm^2	Surface Pressure Π $(\gamma_o - \gamma)$
Perfluorotributylamine/Pluronic F-68				
Water	36.68			
2.5×10^{-6}	30.20	8.621×10^{-13}	192.6	6.48
5×10^{-6}	29.55	3.017×10^{-12}	55.0	7.13
2.5×10^{-5}	26.04	5.172×10^{-12}	32.0	10.64
5×10^{-5}	21.62	6.034×10^{-12}	27.0	15.06
5×10^{-4}	20.13	8.621×10^{-12}	19.26	16.55
Perfluorodecalin/Pluronic F-68				
Water	36.33			
2.5×10^{-6}	36.68	1.466×10^{-11}	11.3	-
5×10^{-6}	26.58	6.897×10^{-12}	24.0	9.75
2.5×10^{-5}	24.96	5.172×10^{-12}	32.0	11.37
5×10^{-5}	17.51	7.759×10^{-12}	21.0	18.82
5×10^{-4}	16.72	8.621×10^{-12}	19.26	19.61
Perfluoromethyldecalin/Pluronic				
Water	62.89			
2.5×10^{-6}	55.36	1.724×10^{-11}	96.3	7.53
5×10^{-6}	49.35	6.034×10^{-12}	27.5	13.54
2.5×10^{-5}	39.80	1.034×10^{-10}	9.67	23.09
5×10^{-5}	22.51	1.034×10^{-10}	9.67	40.38
5×10^{-4}	14.19	6.897×10^{-11}	2.4	48.70

Table 4.1-2 Continued

Molarity of Surfactant Solution	Interfacial tension at 25°C (mNm^{-1})	Surface excess, Γ moles/cm^2	Area per molecule nm^2	Surface Pressure Π ($\gamma_o - \gamma$)
Perfluoromethylcyclohexane/Pluronic F-68				
Water	58.77			
2.5×10^{-6}	57.36	1.379×10^{-11}	12.04	1.41
5×10^{-6}	48.25	2.586×10^{-11}	6.42	10.52
2.5×10^{-5}	47.83	8.621×10^{-11}	1.92	10.94
5×10^{-5}	30.39	9.483×10^{-11}	1.75	28.38
5×10^{-4}	-			
Perfluoro-1,3-dimethylcyclohexane/Pluronic F-68				
Water	67.72			
2.5×10^{-6}	53.82	1.552×10^{-11}	10.7	13.9
5×10^{-6}	51.99	1.379×10^{-11}	12.04	15.73
2.5×10^{-5}	42.12	2.241×10^{-11}	7.4	25.6
5×10^{-5}	35.84	1.379×10^{-10}	1.204	31.88
5×10^{-4}	22.96	8.621×10^{-11}	1.926	44.76
Perfluorohexane/Pluronic F-68				
Water	57.87			
2.5×10^{-6}	17.69	3.448×10^{-12}	48.2	40.18
5×10^{-6}	16.1	8.621×10^{-12}	19.26	41.77
2.5×10^{-5}	16.09	4.31×10^{-12}	38.52	40.78
5×10^{-5}	14.68	1.724×10^{-11}	96.3	43.19
5×10^{-4}	13.95	8.621×10^{-12}	19.26	43.92

Table 4.1-2 Continued

Molarity of Surfactant Solution	Interfacial tension at 25°C (mNm^{-1})	Surface excess, Γ moles/cm^2	Area per molecule nm^2	Surface Pressure Π $(\gamma_o - \gamma)$
Perfluorotributylamine/SDDS				
10^{-4}	42.58	-	-	-
5×10^{-4}	42.5	8.621×10^{-12}	19.26	-
10^{-3}	24.44	1.552×10^{-11}	10.7	12.24
5×10^{-3}	17.14	6.897×10^{-11}	2.41	19.54
10^{-2}	-		-	-
Perfluorodecalin/SDDS				
10^{-4}	69.66	8.621×10^{-13}	192.6	-
5×10^{-4}	68.23	2.586×10^{-11}	64.2	-
10^{-3}	66.08	1.207×10^{-11}	13.7	-
5×10^{-3}	36.15	2.5×10^{-11}	6.6	0.18
10^{-2}	22.15	2.586×10^{-11}	6.4	14.18
Perfluoromethyldecalin/SDDS				
10^{-4}	62.32	1.724×10^{-12}	96.3	0.57
5×10^{-4}	59.77	2.586×10^{-12}	64.0	3.12
10^{-3}	57.31	8.621×10^{-12}	19.3	5.58
5×10^{-3}	31.06	2.155×10^{-11}	7.7	31.83
10^{-2}	18.71	1.767×10^{-10}	9.4	44.18

Table 4.1-2 Continued

Molarity of Surfactant Solution	Interfacial tension at 25°C (mNm^{-1})	Surface excess, Γ moles/cm^2	Area per molecule nm^2	Surface Pressure Π $(\gamma_o - \gamma)$
Perfluoromethylcyclohexane/SDDS				
10^{-4}	67.71	1.724×10^{-12}	96.3	-
5×10^{-4}	-	-	-	-
10^{-3}	63.0	7.759×10^{-12}	21.0	-
5×10^{-3}	50.87	1.465×10^{-11}	11.3	7.9
10^{-2}	36.57	1.983×10^{-11}	8.4	22.2
Perfluoro-1,3-dimethylcyclohexane/SDDS				
10^{-4}	67.89	2.759×10^{-11}	6.0	-
5×10^{-4}	-	1.724×10^{-11}	9.63	-
10^{-3}	35.84	6.896×10^{-11}	2.41	31.88
5×10^{-3}	31.07	5.172×10^{-11}	3.21	36.65
10^{-2}	29.17	2.586×10^{-11}	6.42	37.55
Perfluorohexane/SDDS				
10^{-4}	70.55	6.896×10^{-12}	24.1	-
5×10^{-4}	63.53	1.034×10^{-10}	9.67	-
10^{-3}	32.83	1.810×10^{-11}	9.2	25.04
5×10^{-3}	21.91	3.448×10^{-11}	4.82	35.96
10^{-2}	16.1	1.724×10^{-11}	9.63	41.77

Table 4.1-2 Continued

Molarity of Surfactant Solution	Interfacial tension at 25°C (mNm^{-1})	Surface excess, Γ moles/cm^2	Area per molecule nm^2	Surface Pressure Π $(\gamma_o - \gamma)$
Perfluorotributylamine/Lecithin				
10^{-5}	68.6	4.31×10^{-12}	38.52	-
5×10^{-5}	68.6	8.621×10^{-12}	19.26	-
10^{-4}	42.49	1.164×10^{-11}	14.26	-
2×10^{-4}	40.12	3.448×10^{-11}	4.815	-
Perfluorodecalin/Lecithin				
10^{-5}	72.05	1.724×10^{-11}	9.63	-
5×10^{-5}	70.64	8.621×10^{-12}	19.26	-
10^{-4}	45.59	1.121×10^{-11}	14.8	-
2×10^{-4}	43.13	4.31×10^{-11}	3.85	-
Perfluoromethyldecalin/Lecithin				
10^{-5}	73.54	6.896×10^{-11}	24.1	-
5×10^{5}	66.17	9.052×10^{-12}	18.3	-
10^{-4}	44.03	9.914×10^{-12}	16.75	18.86
2×10^{-4}	43.21	3.017×10^{-11}	5.5	19.68

Table 4.1-2 Continued

Molarity of Surfactant Solution	Interfacial tension at 25°C (mNm^{-1})	Surface excess, Γ moles/cm^2	Area per molecule nm^2	Surface Pressure Π $(\gamma_o - \gamma)$
Perfluoromethylcyclohexane/Lecithin				
10^{-5}	76.44	4.31×10^{-12}	38.5	-
5×10^{-5}	69.56	7.759×10^{-12}	21.4	-
10^{-4}	61.79	1.164×10^{-11}	14.3	-
2×10^{-4}	52.66	1.25×10^{-11}	13.3	6.11
Perfluoro-1,3-dimethylcyclohexane/Lecithin				
10^{-5}	78.67	9.483×10^{-12}	17.5	-
5×10^{-5}	68.63	2.586×10^{-11}	6.42	-
10^{-4}	67.72	7.328×10^{-11}	2.27	0
2×10^{-4}	53.82	1.681×10^{-11}	9.88	13.9
Perfluorohexane/Lecithin				
10^{-5}	58.99	3.017×10^{-11}	5.5	-
5×10^{-5}	53.62	1.293×10^{-11}	12.84	4.25
10^{-4}	53.6	3.879×10^{-11}	4.28	4.27
2×10^{-4}	44.42	5.172×10^{-11}	3.21	13.45

Table 4.1-2 Continued

Molarity of Surfactant Solution	Interfacial tension at 25°C (mNm^{-1})	Surface excess, Γ moles/cm^2	Area per molecule nm^2	Surface Pressure Π ($\gamma_o - \gamma$)
Perfluorotributylamine/F.C. 126				
10^{-4}	29.80	4.31×10^{-12}	38.52	6.88
5×10^{-4}	29.79	1.724×10^{-11}	9.63	6.87
10^{-3}	26.44	5.172×10^{-11}	3.21	10.24
5×10^{-3}	13.61	7.328×10^{-11}	2.27	23.07
10^{-2}	-	-	-	-
Perfluorodecalin/F.C. 126				
10^{-4}	41.07	1.724×10^{-11}	9.63	-
5×10^{-4}	39.01	4.31×10^{-11}	3.82	-
10^{-3}	34.36	6.465×10^{-11}	2.57	1.97
5×10^{-3}	25.75	7.328×10^{-11}	2.27	10.58
10^{-2}	18.34	9.483×10^{-11}	1.75	17.99
Perfluoromethyldecalin/F.C. 126				
10^{-4}	45.69	3.017×10^{-11}	5.50	17.20
5×10^{-4}	39.84	5.603×10^{-11}	2.96	23.05
10^{-3}	31.09	8.190×10^{-11}	2.03	31.8
5×10^{-3}	20.58	4.741×10^{-11}	3.5	42.31
10^{-2}	20.21	8.621×10^{-12}	19.26	42.68

Table 4.1-2 Continued

Molarity of Surfactant Solution	Interfacial tension at 25°C (mNm^{-1})	Surface excess, Γ moles/cm^2	Area per molecule nm^2	Surface Pressure Π $(\gamma_o - \gamma)$
Perfluoromethylcyclohexane/F.C. 126				
10^{-4}	65.05	6.034×10^{-11}	2.75	-
5×10^{-4}	56.82	9.483×10^{-11}	1.75	1.95
10^{-3}	38.45	1.466×10^{-10}	1.13	20.42
5×10^{-3}	23.38	6.466×10^{-11}	2.57	35.39
10^{-2}	20.75	2.586×10^{-11}	6.42	38.02
Perfluoro-1,3-dimethylcyclohexane/F.C. 126				
10^{-4}	44.37	4.31×10^{-12}	38.52	23.35
5×10^{-4}	44.30	2.155×10^{-11}	7.70	23.42
10^{-3}	39.30	7.759×10^{-11}	2.14	28.42
5×10^{-3}	20.43	6.034×10^{-11}	2.75	47.29
10^{-2}	20.42	4.31×10^{-11}	3.85	47.3
Perfluorohexane/F.C. 126				
10^{-4}	53.72	3.017×10^{-11}	5.50	4.15
5×10^{-4}	49.31	3.017×10^{-11}	5.50	8.56
10^{-3}	48.49	6.466×10^{-11}	2.57	9.38
5×10^{-3}	24.94	8.621×10^{-11}	1.93	32.93
10^{-2}	20.22	7.328×10^{-11}	2.27	37.65

Table 4.1-2 Continued

Molarity of Surfactant Solution	Interfacial tension at 25°C (mNm^{-1})	Surface excess, Γ moles/cm^2	Area per molecule nm^2	Surface Pressure Π $(\gamma_o - \gamma)$
Perfluorotributylamine/Monflor 51				
10^{-4}	33.6	1.379×10^{-10}	1.20	3.08
5×10^{-4}	11.16	1.078×10^{-10}	1.55	15.52
10^{-3}	7.66	5.603×10^{-11}	2.96	29.02
5×10^{-3}	3.01	2.586×10^{-11}	6.42	33.67
10^{-2}	3.01	8.621×10^{-11}	1.93	33.67
Perfluorodecalin/Monflor 51				
10^{-4}	26.6	1.034×10^{-11}	16.1	9.73
5×10^{-4}	9.37	6.466×10^{-11}	2.57	26.96
10^{-3}	8.15	8.621×10^{-11}	1.93	28.18
5×10^{-3}	5.42	8.621×10^{-11}	1.93	30.91
10^{-2}	-	-		-
Perfluoromethyldecalin/Monflor 51				
10^{-4}	27.17	9.914×10^{-12}	1.67	35.72
5×10^{-4}	12.29	5.603×10^{-11}	2.96	50.6
10^{-3}	10.97	3.017×10^{-11}	5.5	51.92
5×10^{-3}	2.91	3.448×10^{-11}	4.81	59.98
10^{-2}	-	-		-

Table 4.1-2 Continued

Molarity of Surfactant Solution	Interfacial tension at 25°C (mNm^{-1})	Surface excess, Γ moles/cm^2	Area per molecule nm^2	Surface Pressure Π $(\gamma_o - \gamma)$
Perfluoromethylcyclohexane/Monflor 51				
10^{-4}	21.56	4.741×10^{-11}	3.50	37.21
5×10^{-4}	10.68	5.172×10^{-11}	3.21	48.09
10^{-3}	9.87	8.621×10^{-11}	1.93	48.9
5×10^{-3}	4.11	6.466×10^{-11}	2.57	54.66
10^{-2}	-			-
Perfluoro-1,3-dimethylcyclohexane/Monflor 51				
10^{-4}	25.46	7.759×10^{-11}	2.14	42,26
5×10^{-4}	9.0	7.328×10^{-11}	2.27	58.72
10^{-3}	6.9	5.172×10^{-11}	3.21	60.82
5×10^{-3}	2.57	1.724×10^{-10}	0.963	65.15
10^{-2}	-	-	-	-
Perfluorohexane/Monflor 51				
10^{-4}	24.94	1.552×10^{-11}	10.7	32.93
5×10^{-4}	6.86	8.621×10^{-11}	1.926	51.01
10^{-3}	4.76	3.448×10^{-11}	4.81	53.11
5×10^{-3}	2.36	8.621×10^{-11}	1.93	55.51
10^{-2}	-	-	-	

Table 4.1-2 Continued

Molarity of Surfactant Solution	Interfacial tension at 25°C (mNm^{-1})	Surface excess, Γ moles/cm^2	Area per molecule nm^2	Surface Pressure Π $(\gamma_o - \gamma)$
Perfluorotributylamine/Forafac 1111				
10^{-4}	21.6	1.004×10^{-11}	16.5	15.08
5×10^{-4}	5.31	5.603×10^{-11}	2.96	31.37
10^{-3}	5.30	1.724×10^{-11}	9.63	31.38
5×10^{-3}	4.78	8.621×10^{-11}	1.93	31.9
10^{-2}	3.47	4.31×10^{-11}	3.85	33.2
Perfluorodecalin/Forafac 1111				
10^{-4}	30.44	1.121×10^{-11}	14.8	5.89
5×10^{-4}	12.74	9.914×10^{-11}	1.67	23.59
10^{-3}	8.74	4.741×10^{-11}	3.5	27.59
5×10^{-3}	7.10	1.293×10^{-10}	1.28	29.23
10^{-2}	-	-		-
Perfluoromethyldecalin/Forafac 1111				
10^{-4}	30.83	1.034×10^{-11}	16.1	32.06
5×10^{-4}	14.34	7.759×10^{-11}	2.14	48.55
10^{-3}	10.97	3.448×10^{-11}	4.81	51.92
5×10^{-3}	8.20	1.724×10^{-10}	0.963	54.69
10^{-2}	-	-	-	-

Table 4.1-2 Continued

Molarity of Surfactant Solution	Interfacial tension at 25°C (mNm^{-1})	Surface excess, Γ moles/cm^2	Area per molecule nm^2	Surface Pressure Π $(\gamma_o - \gamma)$
Perfluoromethylcyclohexane/Forafac 1111				
10^{-4}	35.79	1.509×10^{-10}	1.1	22.98
5×10^{-4}	10.06	1.034×10^{-10}	1.61	48.71
10^{-3}	7.63	3.017×10^{-11}	5.5	51.14
5×10^{-3}	7.63	8.621×10^{-11}	1.93	51.14
10^{-2}	-	-		-
Perfluoro-1,3-dimethylcyclohexane/Forafac 1111				
10^{-4}	41.63	1.724×10^{-11}	9.63	26.09
5×10^{-4}	12.19	9.914×10^{-11}	1.675	55.53
10^{-3}	8.96	4.741×10^{-11}	3.5	59.76
5×10^{-3}	2.57	4.31×10^{-11}	3.85	65.15
10^{-2}	-	-	-	-
Perfluorohexane/Forafac 1111				
10^{-4}	5.8	8.621×10^{-12}	19.26	52.07
5×10^{-4}	5.8	6.466×10^{-12}	25.72	52.07
10^{-3}	4.29	4.31×10^{-12}	38.52	52.58
5×10^{-3}	3.11	4.31×10^{-12}	38.52	54.76
10^{-2}	-	-	-	-

4.1.2.1 The effect of Additives on Interfacial tension

A number of interfacial tension measurements were made in the presence of a small concentration of an additive in the oil phase (usually another fluorocarbon chemical). In general, the presence of a small quantity (less than 20 m moles/litre), of the additives investigated, in the oil phase had no effect on the interfacial tension. The system of perfluorohexane-n-hexane was investigated in more detail. The liquid mixtures of these chemicals were made containing various proportions of one liquid in the mixture and the interfacial tension of the liquid-liquid mixtures against solutions of sodium dodecyl sulphate (SDDS) in water were measured. The data are presented in table 4.1.2.1-1 and illustrated in figure 10 . The surface tensions of solutions of perfluorohexane in n-hexane are listed in table 4.1.2.1-2.

Table 4.1.2.1-1 Interfacial tensions of mixtures of n-hexane and Perfluorohexane

Molarity of SDDS solution	Interfacial tension (mNm^{-1}) at 25°C		
	n-hexane	Molarity of Perfluorohexane solution in n-hexane	
		0.4	0.6
10^{-4}	39.1	57.5	32.5
2×10^{-4}	35.0	52.5	30.5
5×10^{-4}	31.7	45.0	27.4
10^{-3}	25.0	30.5	24.0
2×10^{-3}	-	26.1	22.5

Fig.10 Interfacial tension of Pf. hexane - hexane mixtures against SDDS aqueous solutions

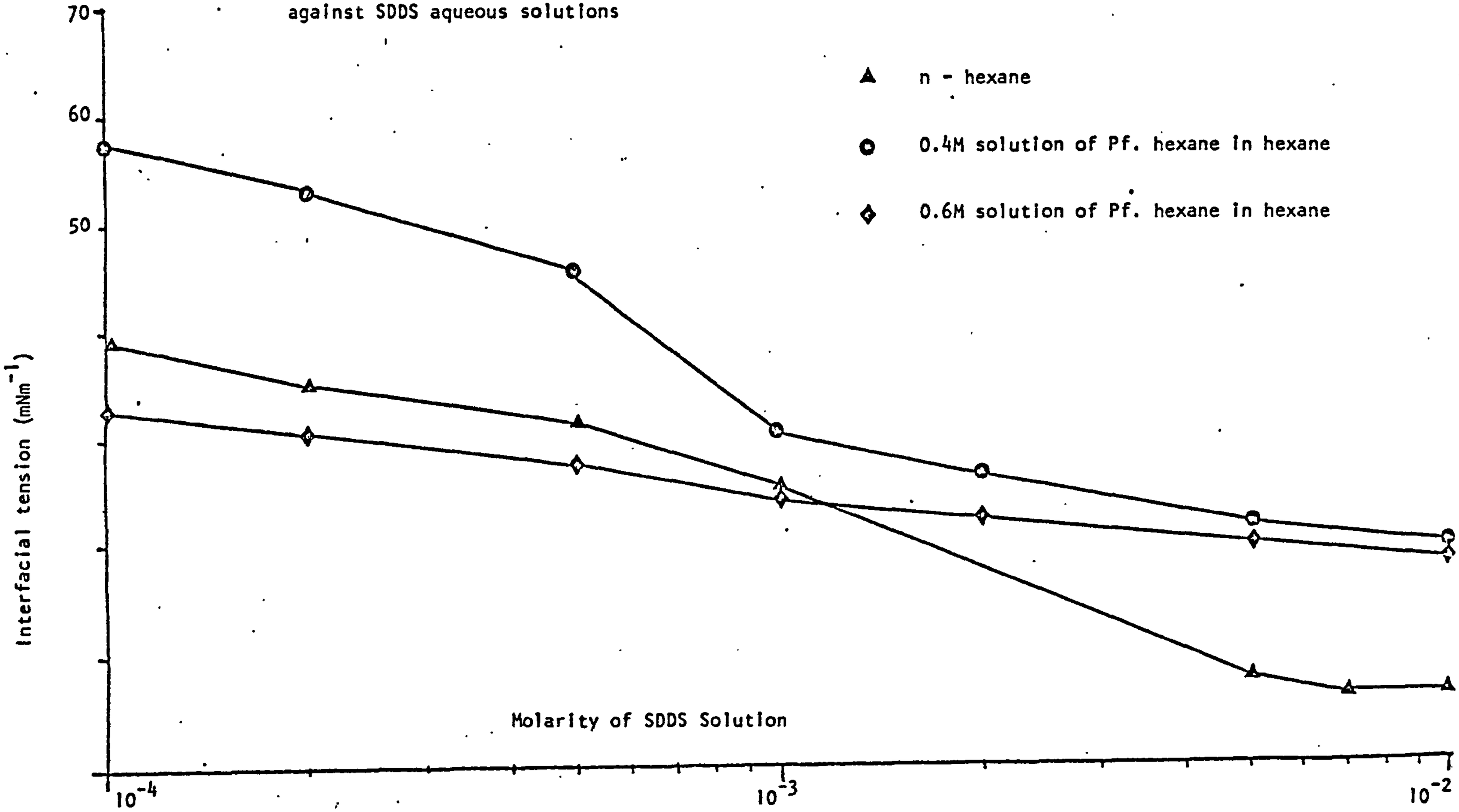


Table 4.1.2.1-1 Continued

Molarity of SDDS solution	Interfacial tension (mNm^{-1}) at 25°C		
	n-hexane	Molarity of Perfluorohexane solution in n-hexane	
		0.4	0.6
5×10^{-3}	7.5	21.5	20.0
7×10^{-3}	6.5	-	-
10^{-2}	6.5	20.0	18.5

Table 4.1.2.1-2 Surface tension of Perfluorohexane Solutions in n-hexane

Molarity of Perfluorohexane Solution in n-hexane	Surface tension (mNm^{-1}) at 25°C
0	18.5
0.1	18.5
0.2	16.5
0.4	13.8
0.6	12.5
0.8	12.5
1.0	12.5

Note that the surface tension of perfluorohexane was measured to be 11.9 mNm^{-1}, at 25°C. The data of table 4.1.2.1-2 show that

the surface tension of n-hexane is decreased on addition of perfluorohexane until a concentration 0.6 moles per litre is reached; further additions of the fluorocarbon has no effect on surface tension. The surface tension showed no measurable change at the perfluorohexane concentration of 0.1 moles per litre or less.

4.2 Single Droplet Stability

All single droplet rest-times represent a mean of at least three measurements.

4.2.1 Data Analysis

A distribution of droplet rest-times was obtained for each system investigated. The results were analysed, where possible, on the basis of a first order kinetics model reported by Davis and Smith (1976).

$$\log N = \frac{-kt}{2.303} + \text{Const.}$$

Where N is the number of drops remaining at time, t, and k is the first order rate constant for coalescence calculated from the gradient of the linear portion of the log N versus t graph, (k = slope x 2.303).

The first order half-life ($T_{\frac{1}{2}} = 0.693/k$) is related to the time for half the droplets to coalesce ($t_{\frac{1}{2}}$) by the following expression:

$$t_{\frac{1}{2}} = T_{\frac{1}{2}} + t_d$$

Where t_d is the film drainage time, see figure 4

It was found that as the overall stability increased the values of $t_{\frac{1}{2}}$ and $T_{\frac{1}{2}}$ became less reproducible. The magnitude of the "drainage time", t_d, appeared to be a poor parameter for comparing different systems, also, it could not be measured accurately, but it was particularly sensitive to an increase in the surfactant concentration and therefore could be utilized to estimate optimum surfactant concentration to obtain maximum stability.

The mathematical techniques of analysis have been compared with the graphical method by Aitchinson and Brown (1963) who have concluded that an experienced user of the log-probability chart could fit data visually, with only slight loss of accuracy, provided that the scatter of the experimental points was small.

4.2.2 The Effect of the Oil Phase and the Emulsifier

The single droplet stability of six fluorocarbon chemicals was measured against an aqueous phase containing a surfactant at a given concentration. Three surfactants were used in this study.

To see the effect of the emulsifier, the single droplet stability of perfluorotributylamine droplets was measured against a number of aqueous phases containing different surface active agents at similar concentrations.

Table 4.2.2-1 lists the single droplet rest-time data for different oils against a given aqueous phase containing the surfactant. The numbers of droplets not coalesced, expressed as a percentage, at time, t, are listed. Fig.11 shows typical graphs drawn from this data.

Figure 11 The coalescence of fluorocarbon oil droplets at the surfactant solution/oil Interface.

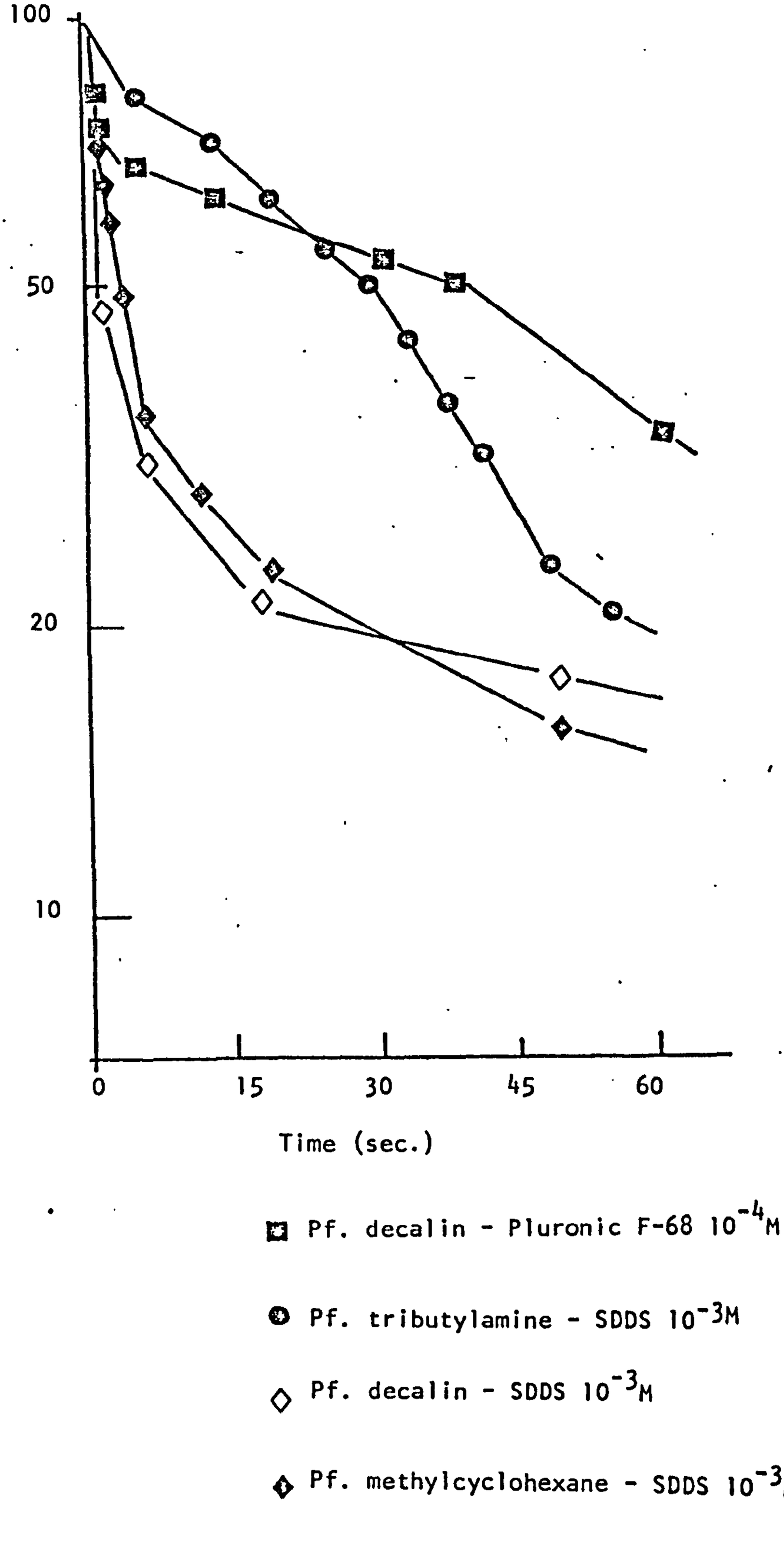


Table 4.2.2-2 lists the single droplet life-times of perfluorotributylamine oil phase against a number of aqueous phases containing different surfactants.

From these data the parameters of $t_{\frac{1}{2}}$, $T_{\frac{1}{2}}$ and t_d were calculated and listed in tables 4.2.2-3 and 4.2.2-4.

It is clear that the nature of the oil phase and the nature of the surfactant both play an important role in droplet stability. When the surfactant is SDDS, the droplet life-times are in the order: Perfluorodecalin < perfluoromethylcyclohexane < Perfluoromethyldecalin < Perfluorotributylamine; and for a given oil the droplets stabilized by the non-ionic surfactant (Pluronic F-68) are much more stable than any of the other surfactants that were studied, (including fluorinated surfactants) even though the concentration of Pluronic F-68 was a thousand fold less than the other surfactants. A tenfold reduction in the concentration of SDDS leads to a corresponding decrease in the stability of perfluorotributylamine droplets.

Table 4.2.2-1 Single droplet stability: the effect of the oil phase and the emulsifier

Oil	Pluronic F-68 10^{-6} M Solution							
Perfluorotributylamine	Time (Sec)	0	15	30	60	120	240	300	600
	No. not coalesced	100	100	82	76	65	29	17	0
Perfluoromethyldecalin	Time (Sec)	0	5	10	20	30	40		
	No. not coalesced	100	100	79	25	12	0		
Perfluoromethyl-cyclohexane	Time (Sec)	0	1	2	5	10	20	34	
	No. not coalesced	100	100	85	57	25	2	0	
Perfluorodecalin	Time (Sec)	0	1	2	5	10	15	20	
	No. not coalesced	100	100	82	48	13	3	0	

Table 4.2.2-1 Continued

Oil	Pluronic F-68 10^{-6} M Solution								
Perfluoro-1,3-dimethyl cyclohexane	Time (Sec)	0	5	10	20	50	100	200	300
	No. not coalesced	100	100	94	86	47	30	9	0
Perfluorohexane (99% mixed isomers) (85% of n-isomer)	Time (Sec)	0	1	2	5	10	20	30	40
	No. not coalesced	100	96	74	60	36	15	4	0
Oil Perfluoro-	Lecithin 10^{-4} M Solution								
Tributylamine	Time (Sec)	0	1	3	5	10	25	50	100	150
	No. not coalesced	100	95	51	35	26	20	14	8	0

Table 4.2.2-1 Continued

Oil Perfluoro-	Lecithin 10^{-4} M Solution								
Methyldecalin	Time (Sec)	0	1	2	5	10	20	40	65	90
	No. not coalesced	100	92	82	64	43	25	15	10	0
Decalin	Time (Sec)	0	1	2	2.5	3	4	7		
	No. not coalesced	100	29	34	13	4	1	0		
Methylcyclohexane	Time (Sec)	0	1	2	3	5	7	10		
	No. not coalesced	100	69	34	19	10	5	0		
1,3-dimethyl-cyclohexane	Time (Sec)	0	1	2	3	5	7			
	No. not coalesced	100	74	31	11	5	2			

Table 4.2.2-1 Continued

Oil Perfluoro-	Lecithin 10^{-4} M Solution								
Hexane	Time (Sec)	0	1	5	10	25	50	70	100
	No. not coalesced	100	82	58	51	44	27	22	0
Oil Perfluoro-	S.D.D.S. 10^{-3} M Solution								
Methyldecalin	Time (Sec)	0	9	10	15	20	25	30	60	80
	No. not coalesced	100	100	99	84	61	47	35	9	3
Decalin	Time (Sec)	0	1	2	5	10	20	50	100	
	No. not coalesced	100	74	38	27	24	20	14	9	

Table 4.2.2-1 Continued

Oil Perfluoro-	S.D.D.S. 10^{-3} M Solution								
Tributylamine	Time (Sec)	0	5	10	15	20	30	45	65	100
	No. not coalesced	100	97	69	62	51	42	28	17	8
Methylcyclohexane	Time (Sec)	0	1	2	3	5	10	20	50	100
	No. not coalesced	100	91	77	63	47	42	34	19	15
Dimethylcyclohexane	Time (Sec)	0	5	10	20	40	66	100	150	180
	No. not coalesced	100	96	92	78	53	33	22	16	0
Hexane	Time (Sec)	0	1.5	3	6	15	30	50	75	120
	No. not coalesced	100	96	89	85	66	59	40	32	0

Table 4.2.2-2 Single Droplet Stability: the effect of the emulsifier

Aqueous Phase	Oil : Perfluorotributylamine									
Monflor 51 10^{-4} M	Time (Sec)	0	0.5	1	1.5	2	2.5	3.5	8	
	No. not coalesced	100	100	96	34	7	2	1	0	
F.C. 170 10^{-4} M	Time (Sec)	0	0.5	1	2	3	5	10	15	20	30
	No. not coalesced	100	100	64	48	42	35	15	4	2	0
Monflor 52 10^{-4} M	Time (Sec)	0	2	2.5	10	25	40	60	100	200	
	No. not coalesced	100	100	99	87	70	45	27	9	1	
Monflor 53 10^{-4} M	Time (Sec)	0	0.5	1	1.5						
	No. not coalesced	100	64	1	0						

Table 4.2.2-2 Continued

Aqueous Phase	Oil : Perfluorotributylamine								
F.C. 126 10^{-4} M	Time (Sec)	0	0.5	1	2	5	7	10	12	15
	No. not coalesced	100	100	96	81	52	32	5	1	0
S.D.D.S. 10^{-4} M	Time (Sec)	0	0.5	1	2	5	10	12	16	20
	No. not coalesced	100	97	69	51	20	8	5	2	0
Forafac 1032 10^{-4} M	Time (Sec)	0	20	50	100	150	200	300		
	No. not coalesced	100	100	72	31	12	3	0		
Lecithin 10^{-4} M	Time (Sec)	0	1	3	5	10	25	50	100	150
	No. not coalesced	100	95	51	35	26	20	14	8	0

Table 4.2.2-2 Continued

Aqueous Phase	Oil : Perfluorotributylamine							
Pluronic F-68 10^{-6} M	Time (Sec)	0	15	30	60	120	240	300	600
	No. not coalesced	100	100	82	76	65	29	17	0

Table 4.2.2-3 Single droplet stability of fluorocarbon oils (25°C)

Oil Phase Perfluoro-	Aqueous Phase								
	Pluronic F-68 10^{-6} M			Lecithin 10^{-4} M			S.D.D.S. 10^{-3} M		
	$t_{\frac{1}{2}}$ (Sec)	$T_{\frac{1}{2}}$	t_d (Sec)	$t_{\frac{1}{2}}$ (Sec)	$T_{\frac{1}{2}}$	t_d (Sec)	$t_{\frac{1}{2}}$ (Sec)	$T_{\frac{1}{2}}$	t_d (Sec)
tributylamine	163.6	147.6	16	3.0	2.8	0.2	30.5	30	0.5
decalin	4.5	3.5	1.0	1.5	1.5	0	2	2	0
methyldecalin	15.7	10.0	5.7	8.0	8.0	0	24	18	6
methylcyclohexane	5.7	4.6	1.1	1.2	1.0	0.2	3.2	3.2	0
1,3-dimethylcyclohexane	61	55	6	1.5	1.0	0.5	42.5	39.5	3
hexane	8.0	7.6	0.4	10.0	10.0	0	37	37	0

Table 4.2.2-4 Single droplet stability : Effect of surfactant

Surfactant in the aqueous phase 10^{-4} M	Oil phase : Perfluorotributylamine		
	$t_{\frac{1}{2}}$ (Sec)	$T_{\frac{1}{2}}$ (Sec)	t_d (Sec)
Monflor 51	1.2	0.8	0.4
Monflor 52	26.0	23.8	2.2
F.C. 170	1.7	1.7	0
F.C. 126	5.0	4.6	0.4
Forafac 1023	2	2	0
Forafac 1032	72	52.7	19.3
Forafac 1111	41	31.5	9.5
SDDS	2.0	2.0	0
Lecithin	3.0	2.8	0.2
Pluronic F-68 (10^{-6} M)	163.6	147.6	16

4.2.3 The effect of additives

The droplet rest-times of fluorocarbon oils, which contained another fluorochemical at a concentration of 20 milimoles per litre, were not significantly different from the droplet rest-times of pure fluorocarbon oil phases. However, solutions of perfluorohexane in n-hexane did show significant differences between single droplet rest-times depending on the concentration of the perfluorohexane in the liquid mixture. The results are illustrated in Fig.12 , and the values of $T_{\frac{1}{2}}$, $t_{\frac{1}{2}}$ and t_d obtained

from these graphs are given in table 4.2.3-1. The aqueous phase was 5×10^{-3} M SDDS solution.

Table 4.2.3-1 The stability of single droplets of Perfluorohexane solutions in n-hexane

Molarity of Perfluorohexane solution in n-hexane	$t_{\frac{1}{2}}$ (Sec)	$T_{\frac{1}{2}}$	t_d (drainage time) (Sec)
0	270	150	120
0.1	21	21.0	0
0.2	212	125.5	87.5
0.4	328	203.0	125.0
0.6	520	332.0	187.5

The n-hexane used in these experiments was specially purified to be better than 99.9% pure, (GLC analysis).

The data show that at smaller concentrations the addition of perfluorohexane to n-hexane leads to a decreased stability of the single oil droplets. But at higher concentrations (0.4 and 0.6 molar solutions) the addition of perfluorohexane leads to an enhanced droplet stability. This may be caused by increased density of perfluorohexane solutions in n-hexane. Therefore the density of these solutions was measured by the specific gravity bottle method. The results are given in table 4.2.3-2.

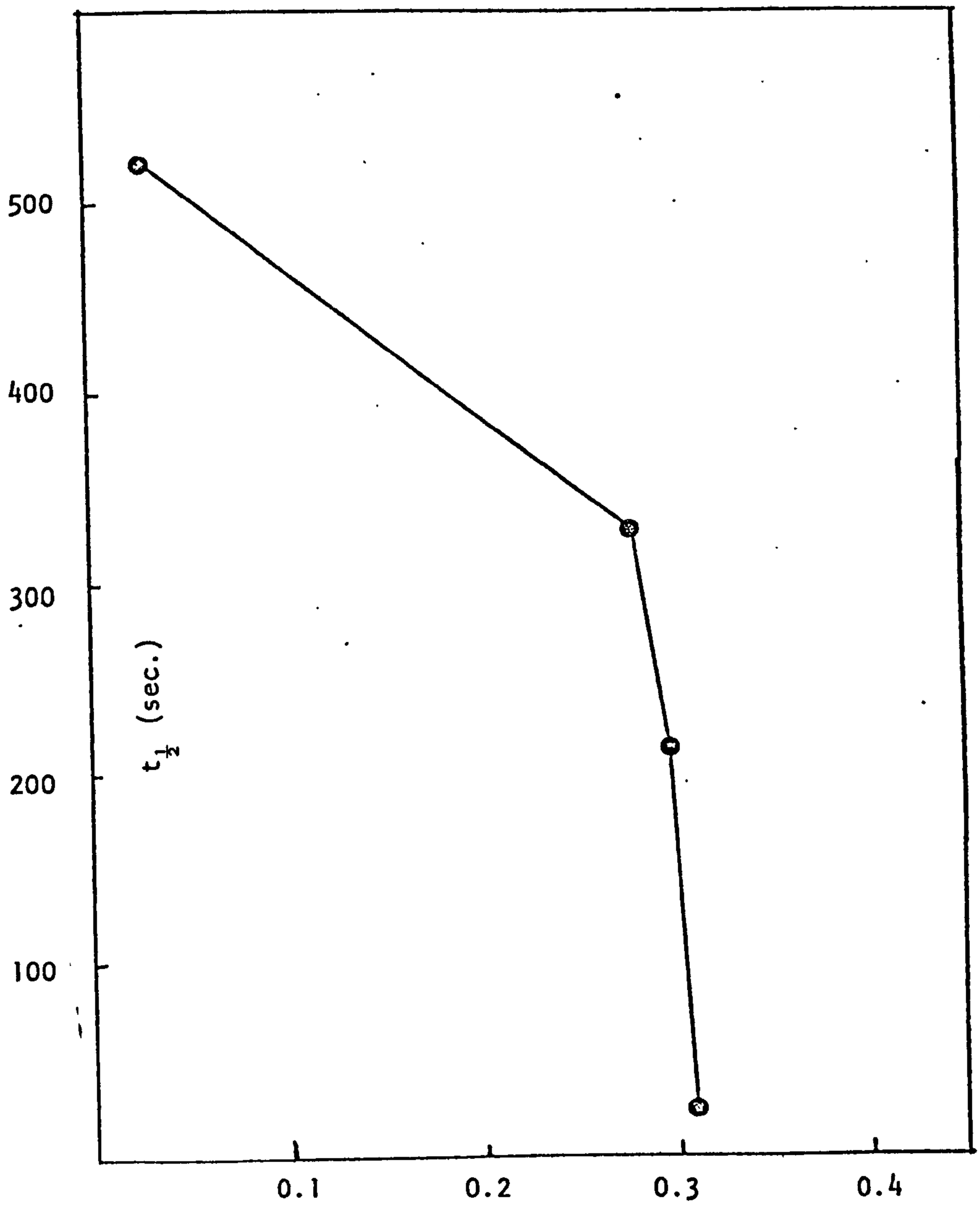
Table 4.2.3-2 Single droplet stability: the effect of additives: the density of Perfluorohexane solutions in n-hexane

Molarity of Perfluorohexane solution in n-hexane	Density at 25°C (g/cm^3)
0	0.65
0.1	0.69
0.2	0.7
0.4	0.72
0.6	0.97

Figure13 illustrates how the single droplet stability parameter, $t_{\frac{1}{2}}$, (the time taken for 50% of the droplets to coalesce), varies with the density difference between the oil phase and the aqueous phase. It is apparent that as the density difference increases the droplet stability decreases. In these experiments the oil phase was lighter and therefore the droplets were rising up to the interface and coalescing. As the density difference decreased due to the addition of perfluorohexane, the upward force exerted by the droplet at the interface would be less. Consequently the interfacial film between the droplet and the oil phase would take longer to drain, leading to extended droplet life-time at the interface.

Figure13 Single droplet Stability.

The Effect of density difference between Phases on $t_{\frac{1}{2}}$ of Perfluorohexane - hexane binary mixtures.



4.3 Bulk Emulsion Stability

The bulk emulsion stability was measured by determining particle size distributions, at suitable time intervals. Two methods of determining the particle size distribution were compared, namely, electronmicroscopy, and stepwise centrifugation as reported by Fujita et al (1971) and Yokoyama et al (1974).

4.3.1 Electronmicroscopy vs. centrifugation as methods for determining particle size distribution

An oil-in-water emulsion was prepared using perfluorotributylamine as the oil phase and Pluronic F-68 as the emulsifier. The droplet size distribution of this emulsion was determined by electronmicroscopy as described in subsection 3.3.2.1 and centrifugation and gas chromatography as described in subsection 3.3.2.2.1.

The particle size distribution data obtained from the two methods are shown in table 4.3.1-1 and plotted in Fig.14

It is clear from the data that both methods are in good agreement regarding the particle size distribution. But electronmicroscopy was chosen as the method for size analysis because it did not involve any dilution of the sample and it was less cumbersome.

Figure 14 Showing results of Particle size analysis by centrifugation and Electromicrography

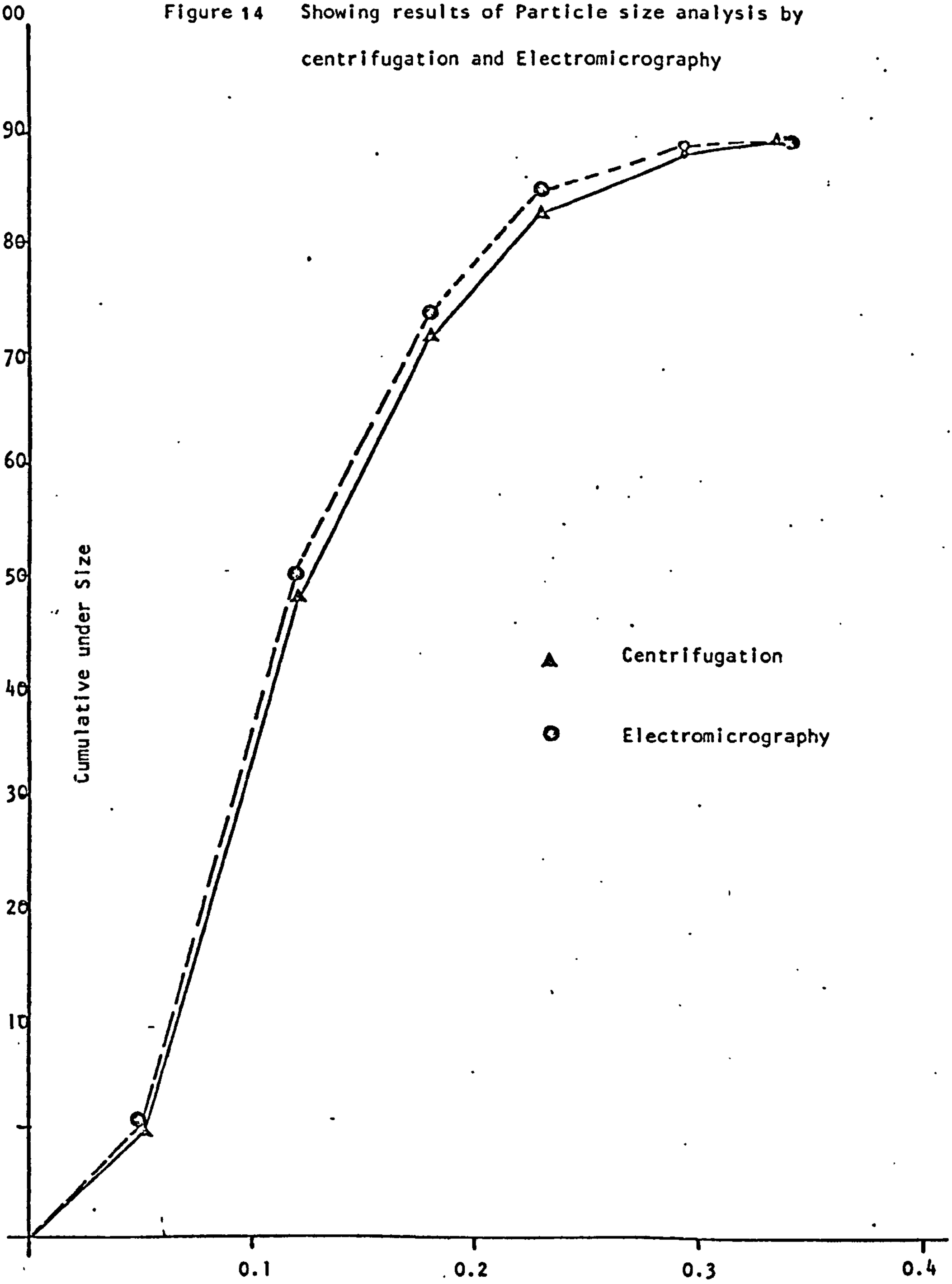


Table 4.3.1-1 Particle size distribution of a Fluorocarbon emulsion obtained by two different methods

Particle Size (μ)	Cumulative percent under size	
	Electronmicroscopy	Centrifugation
0.05	10	10
0.121	60	58
0.176	84	82
0.232	95	93
0.287	99	99
0.342	100	100

4.3.2 The effect of the oil phase and the emulsifier

Emulsions containing different fluorocarbon oil phases were prepared, containing the same emulsifier and the same phase volumes. The emulsions were stored in clear glass medicine bottles at room temperature and particle size distributions were measured at various time intervals. Similarly emulsions of a given oil were investigated containing different emulsifiers.

4.3.2.1 Droplet size measurements

The droplet size measurements were carried out by electronmicroscopy. Typical electronmicrographs are shown in Plates 1 and 2.

The droplet size distribution plots show changes in the number percent versus droplet diameter curves as the emulsions age. An example is illustrated in Fig.15 . Table 4.3.2-1 lists the mean particle diameter of emulsions with ageing at room temperature,

PLATE 1

Electronmicrographs of Fluorocarbon Emulsions.

Bar = one micron in each case.

A. Perfluorotributylamine 10% w/v emulsion stablilised with 4% w/v Pluronic F-68.

B. Perfluorodecalin 10% w/v emulsion stabilised by 4% w/v lecithin.

C. Perfluorodecalin 10% w/v emulsion stabilised by 4% w/v SDDS.

D. Perfluorodecalin 10% w/v emulsion stabilised by 4% w/v lecithin at a higher magnification.

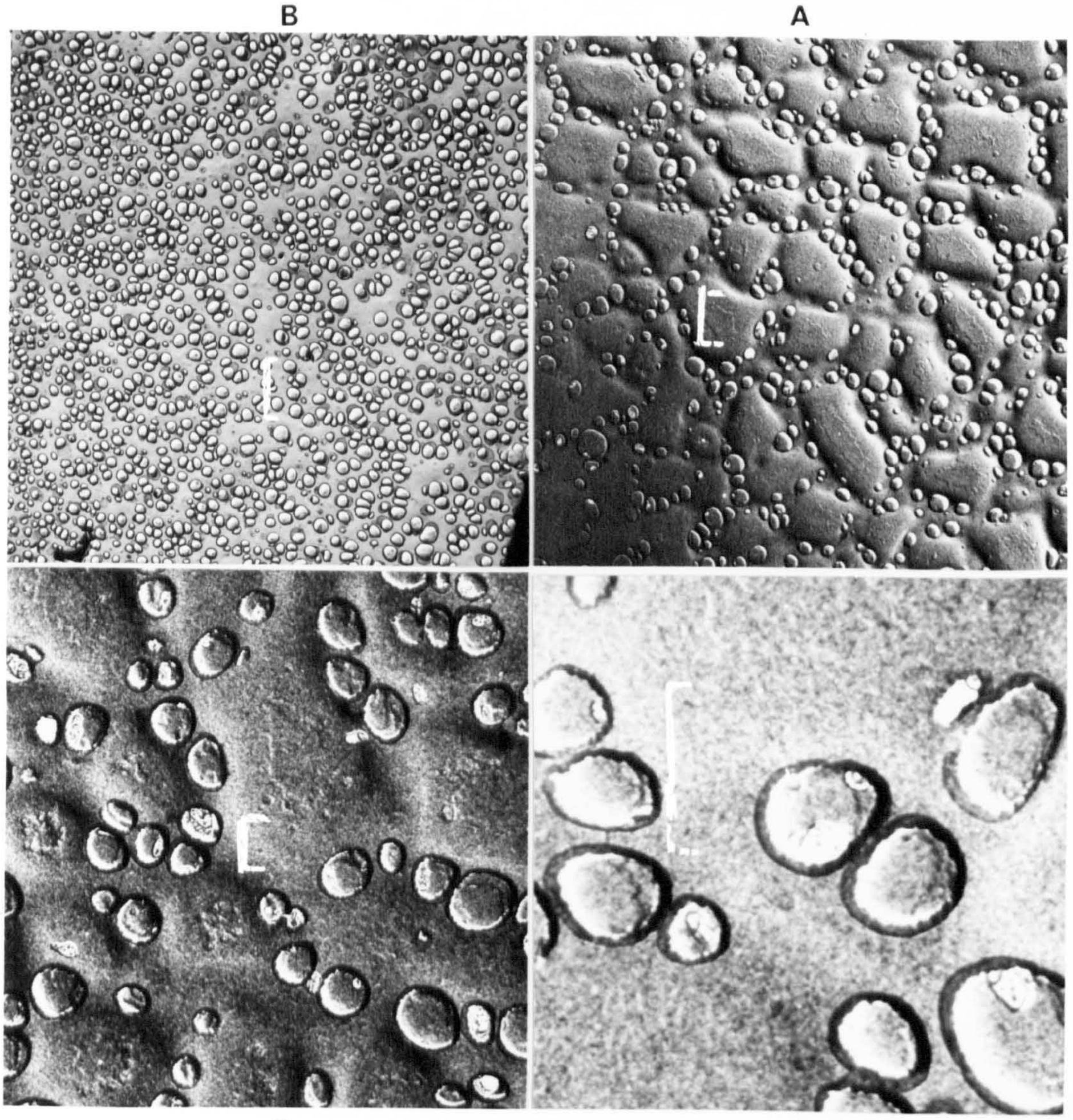


PLATE 1

PLATE 2

Electronmicrographs of Fluorocarbon Emulsions.

Bar = one micron in all cases.

A. Perfluorotributylamine 10% w/v emulsion stabilised by 4% w/v Pluronic F-68, after 36 days storage.

B. Perfluorodecalin 10% w/v emulsion stabilised by 4% w/v lecithin, after 36 days storage.

C. Perfluoromethylcyclohexane 10% w/v emulsion stabilised by 4% w/v lecithin, after 36 days storage.

D. Perfluorotributylamine 10% w/v emulsion stabilised by 4% w/v Pluronic F-68, initial particle size.

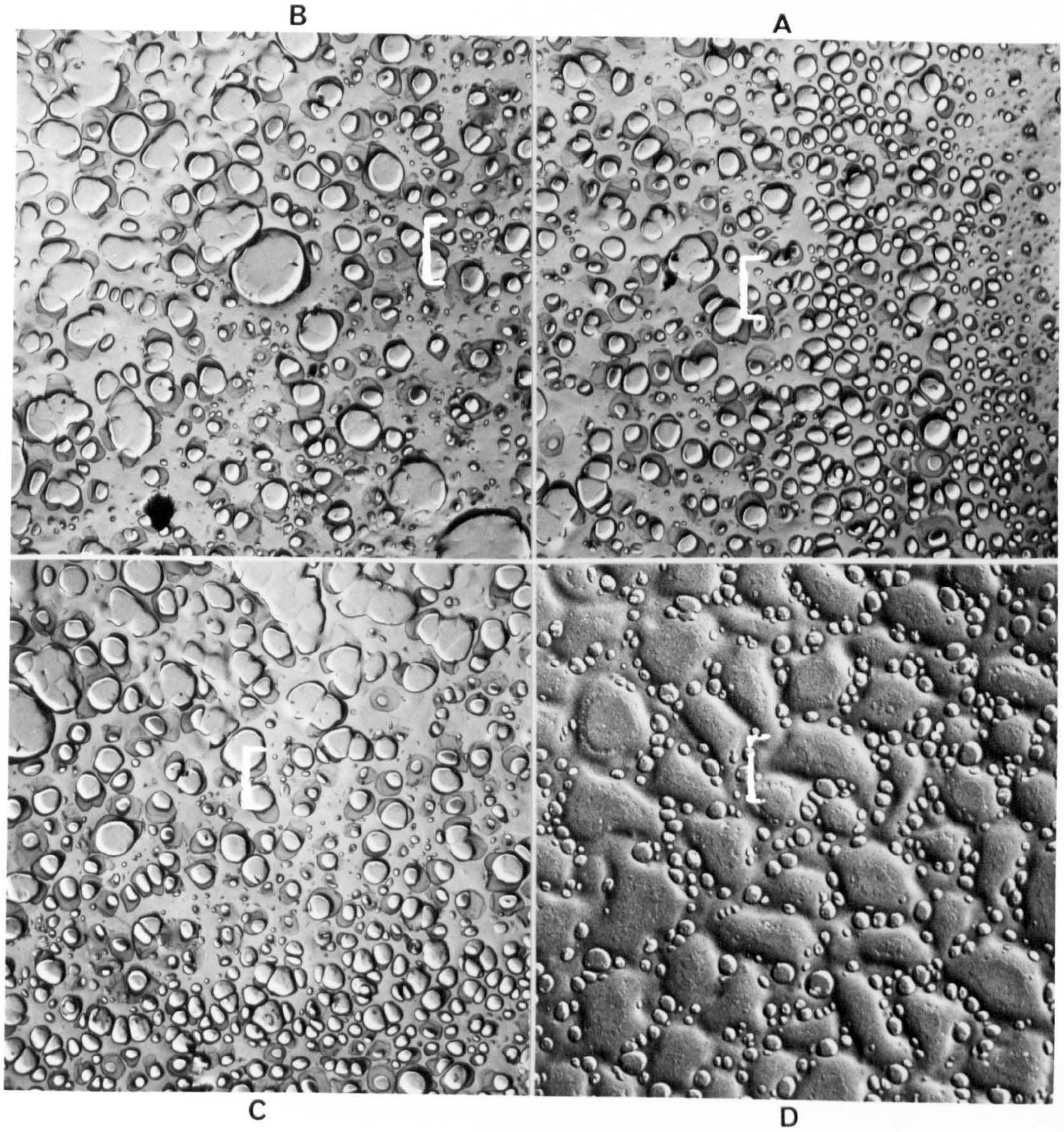
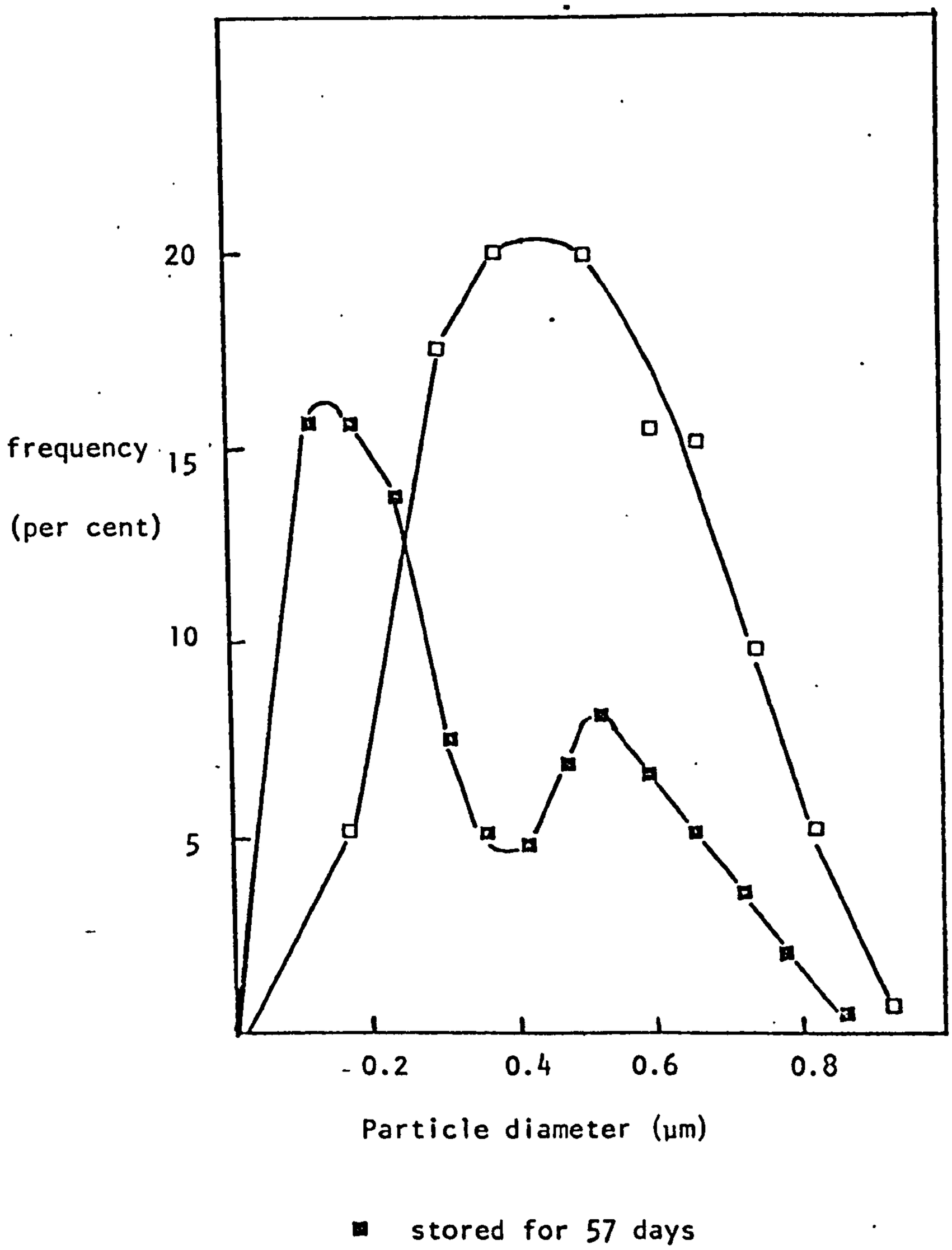


PLATE 2

Figure 15 The Effect of Storage on the Particle size distribution of a perfluoromethylcyclohexane emulsion stabilised by Pluronic F-68.



for three different perfluorochemical oils and four different surfactants. The data in table 4.3.2-2 show the changes in the mean particle size of perfluorotributylamine emulsions stabilized by different surfactants.

4.3.2.2 Viscosity of the emulsions

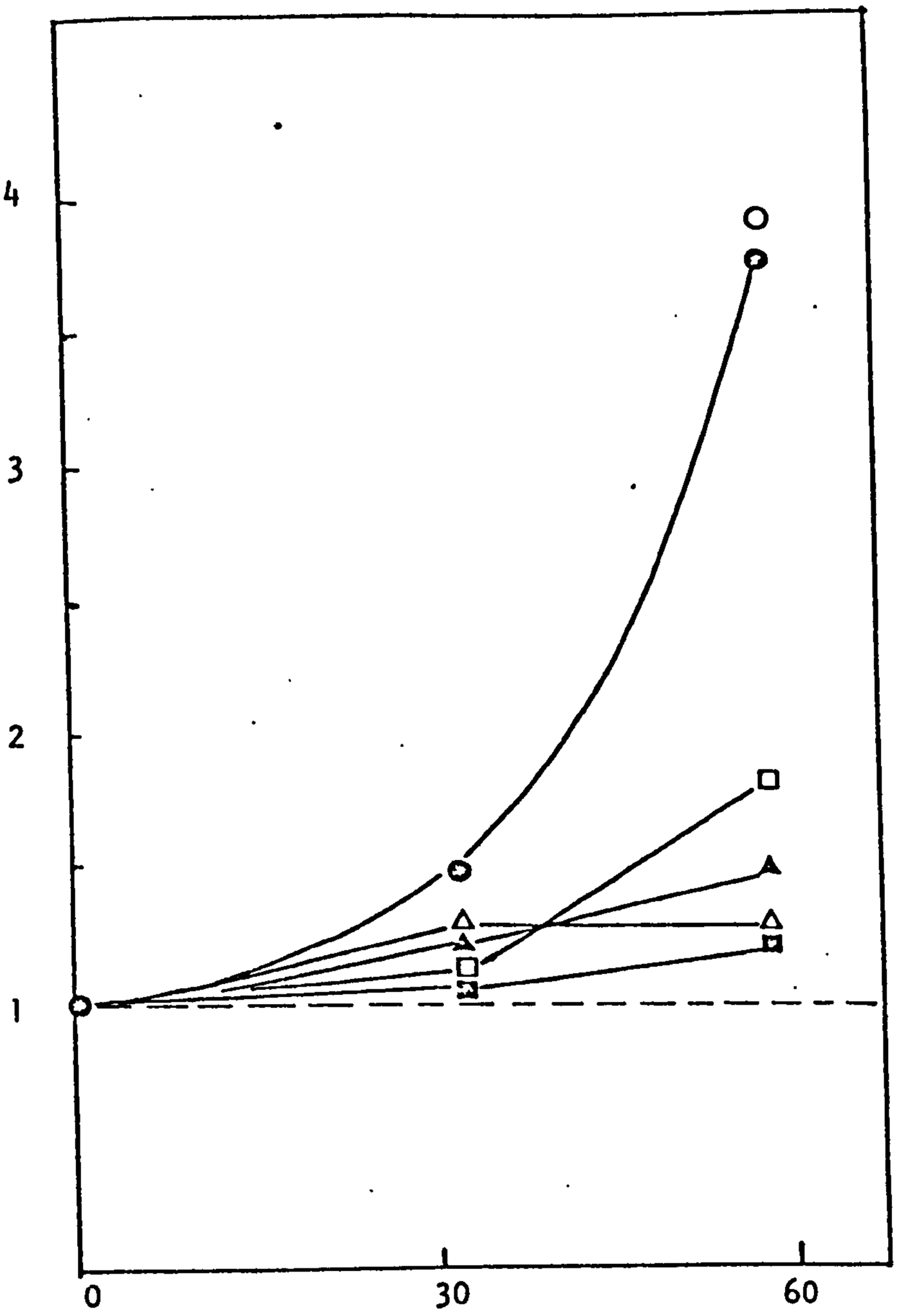
The relative viscosities of the emulsions changed little on storage. The time span during which there was negligible change in viscosity was taken as a stability index. After about 15 days emulsions exhibited a high relative viscosity. This was caused by sedimentation of the larger emulsion particles in the U-tube capillary. The sediment was quite visible to the naked eye. Table 4.3.2-3 gives the time span without significant change in viscosity of various emulsion systems.

The results suggest that perfluorotributylamine forms relatively stable emulsions with all the surfactants studied, but Perfluorodecalin and Perfluoromethylcyclohexane form relatively stable emulsions only when Pluronic F-68 or the fluorinated surfactant mixture is used as emulsifier. Perfluorodecalin appears to form the least stable emulsions out of the three Perfluorochemicals investigated.

In general, it was found that increasing the surfactant concentration retarded the coarsening of the emulsion. This effect was more noticeable in the case of SDDS than Pluronic F-68.

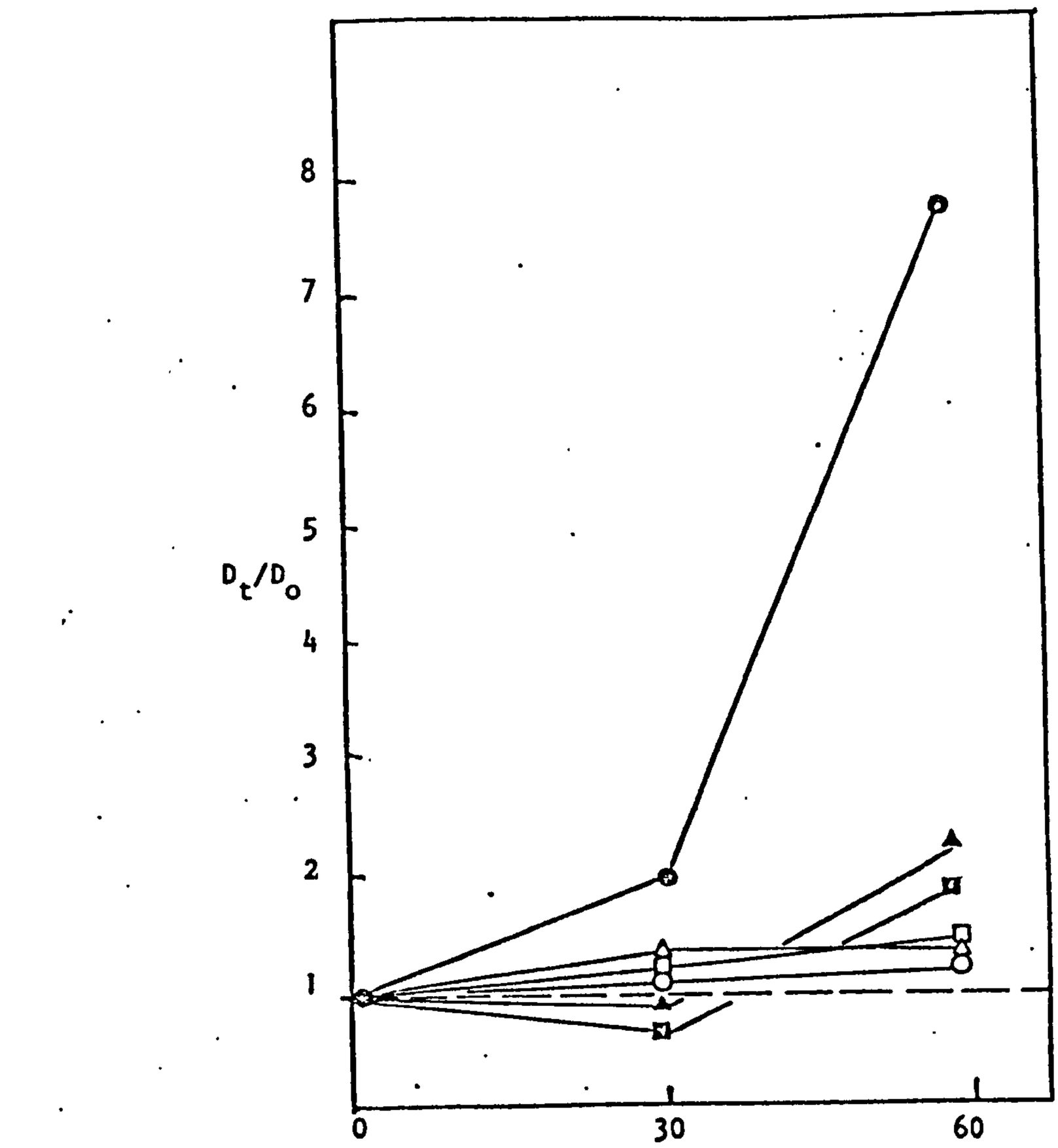
Figures16,17and18 illustrate the effect of the oil phase and the emulsifier respectively. In these illustrations the mean particle size data have been plotted using the normalizing function D_t/D_o;

Figure 16 The Effect of storage on the mean droplet diameter of fluorocarbon emulsions stabilised by SDDS and egg lecithin.



	●	Pf. decalin	○	
SDDS	■	Pf. tributylamine	□	Lecithin
	▲	Pf. methylcyclohexane	△	

Figure 17 The Effect of Storage on the mean droplet diameter of fluorocarbon emulsions stabilised by Pluronic F-68 and a mixed fluorinated surfactant system.



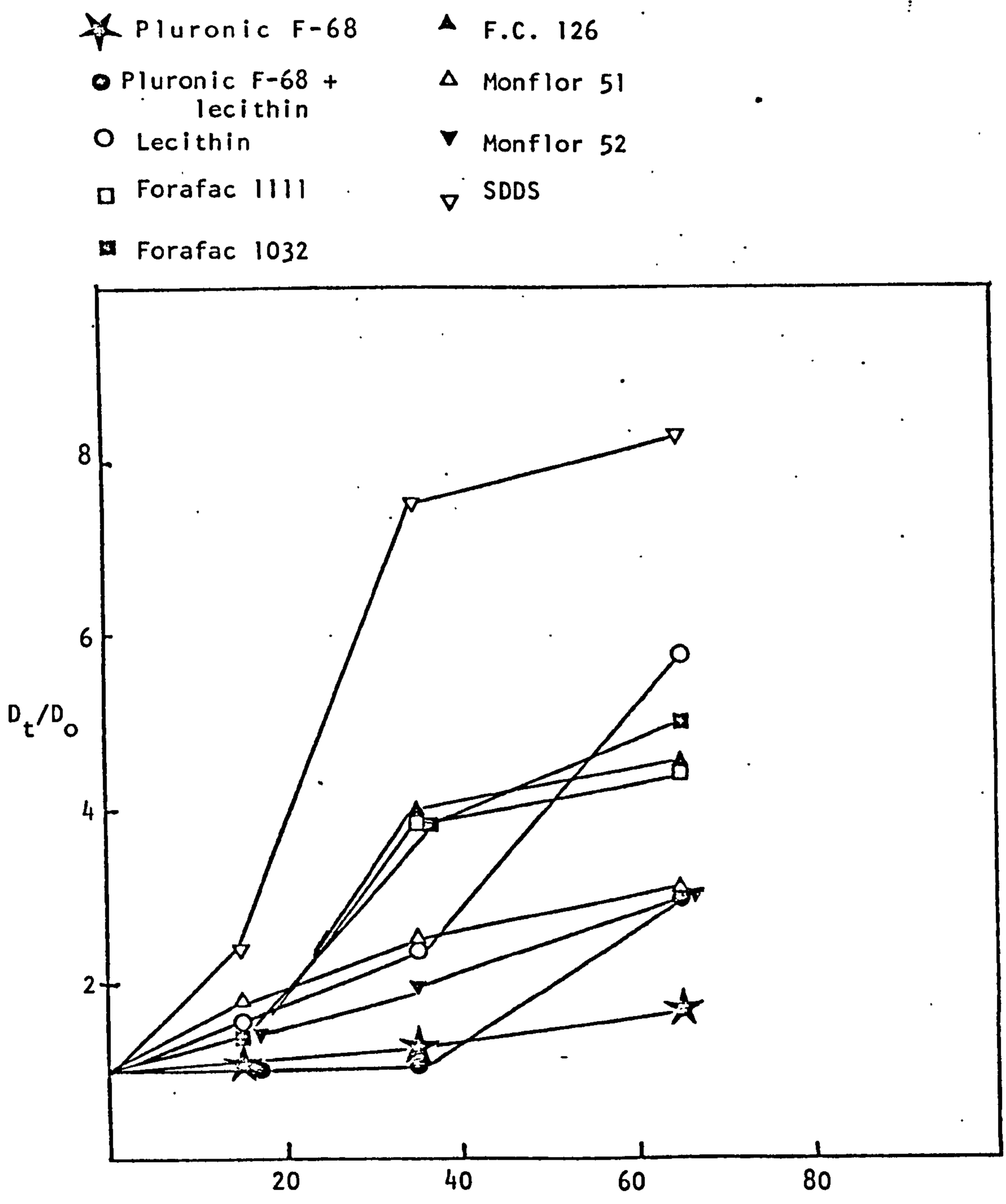


Figure 18 The bulk emulsion stability:

The effect of the Surfactant on the mean droplet diameter on ageing of emulsion.

where D_t is the mean diameter at storage time, t, and D_o is the initial particle size.

Considering first the initial size of the droplets we find that for a given oil the mean sizes are affected by the emulsifier, F.C. 126 = Forafac 1111 = Forafac 1032 < Lecithin + Pluronic F-68 < SDDS < lecithin = Monflor 51 < Monflor 52 < Pluronic F-68. For a given surfactant the mean sizes are affected by the oil phases; Perfluorodecalin < Perfluoromethylcyclohexane and Perfluorotributylamine, except for SDDS as emulsifier where the order is reversed. Upon storage for a given oil the mean size is affected by the surfactant; Pluronic F-68 + lecithin < Pluronic F-68 < Monflor 52 = Monflor 51 < Forafac 1111 = F.C. 126 < Forafac 1032 < lecithin < SDDS. For a given surfactant the mean sizes are affected by the oil phase; Perfluorotributylamine < Perfluoromethylcyclohexane ≪ Perfluorodecalin.

Emulsions produced using Pluronic F-68 and any of the fluorochemical oils were most stable on storage except for the combined emulsifier system, (Pluronic F-68 and lecithin), which gave better stability up to 36 days storage, but later the emulsion became less stable which was attributed to degradation of lecithin by microorganisms; sterile emulsions did not show the reduced stability. Perfluorodecalin and lecithin gave fine particle sized emulsions initially.

The data for the emulsions emulsified using the fluorocarbon surfactant mixture demonstrates an interesting effect; the mean size decreases on storage and then increases rapidly.

The distributions of particles obtained for each emulsion system did not follow any particular distribution function (e.g. normal, log normal) and in some cases (particularly oils emulsified with SDDS and fluorinated surfactant mixture) the particle size distribution was bimodal in nature.

It is apparent from the data that the nature of the oil phase and surfactant has profound effect on the initial particle size and stability of fluorochemical emulsion systems.

Table 4.3.2-1 The effect of storage on mean particle diameter of Perfluorochemical emulsions

* 2% w/v each of F.C. 126 and Forafac 1111

Oil (10% w/v)	Surfactant (4% w/v)	Diameter (μm)		
		Day/ 0	Day 32	Day 57
Perfluoromethyl-cyclohexane	Pluronic F-68	0.41	0.44	0.51
	SDDS	1.62	1.94	2.25
	*Fluorinated surfactants	0.63	0.51	1.38
	Lecithin	0.43	0.56	0.57
Perfluorodecalin	Pluronic F-68	0.40	0.41	0.48
	SDDS	0.48	0.70	1.77
	*Fluorinated surfactants	0.31	0.52	2.28
	Lecithin	0.23	-	0.86
Perfluorotributylamine	Pluronic F-68	0.45	0.48	0.59
	SDDS	0.60	0.67	0.74
	*Fluorinated surfactants	0.57	0.40	1.14
	Lecithin	0.41	0.46	0.73

Table 4.3.2-2 Stability of Perfluorochemical emulsions: the effect of the surfactant

*2% w/v of each

Oil (20% w/v)	Surfactant (4% w/v)	Diameter (μm)			
		Day 0	Day 15	Day 36	Day 66
Perfluorotributylamine	Pluronic F-68	0.34	0.40	0.44	0.61
	Lecithin	0.26	0.41	0.62	1.5
	*Lecithin and Pluronic F-68	0.20	0.20	0.24	0.60
	Monflor 51	0.26	0.46	0.64	0.81
	F.C. 126	0.18	0.21	0.73	0.83
	Forafac 1111	0.18	0.21	0.71	0.82
	Forafac 1032	0.18	0.26	0.71	0.91
	SDDS	0.25	0.60	1.91	2.08
	Monflor 52	0.33	0.47	0.66	1.07

Table 4.3.2-3 Viscosity of Perfluorochemical emulsions

*2% w/v each of F.C. 126 and Forafac 1111

Surfactant	Time (days) without viscosity change		
	Oil		
	Pf. tributylamine	Pf. decalin	Pf. methyl-cyclohexane
SDDS	7	0	0
Pluronic F-68	6	0	0
Lecithin	13	6	13
*Fluorinated Surfactants	13	7	13

4.3.2.3 Forced coalescence

In order to find out if forced coalescence in a centrifuge, can be used to predict the bulk stability of fluorocarbon emulsions, a number of fluorocarbon emulsions were centrifuged in an M.S.E. "High Speed" centrifuge at 7.4×10^4 g (where g is the force due to gravity).

The effect of the oil phase and the emulsifier was investigated. The volume of the oil which had separated after centrifugation was accurately measured. Table 4.3.2.3-1 lists data showing the effect of the oil phase and table 4.3.2.3-2 gives the data to show the effect of the emulsifier.

Table 4.3.2.3-1 Emulsion stability: forced coalescence; the effect of the oil phase

Oil Phase 20% w/v	Surfactant 4% w/v	Volume of oil separated after centrifugation at 7.4×10^4 g for one hour
Perfluoro-tributylamine	Pluronic F-68	5.7 ml
decalin		6.8 ml
methyldecalin		5.9 ml
methylcyclohexane		6.6 ml
1,3-dimethylcyclo-hexane		6.3 ml
hexane		5.9 ml

Table 4.3.2.3-2 Emulsion stability: forced coalescence; the effect of the emulsifier

Surfactant 10^{-2} M	Oil Phase 20% w/v	Volume of oil separated (ml) after centrifugation at 7.4×10^4 g for one hour
Monflor 51	Perfluorotributylamine	0
Monflor 52		0
Monflor 53		6
F.C. 126		0.2
F.C. 170		0
Forafac 1032		0
Forafac 1111		0
Forafac 1023		0
Pluronic F-68		5.7
Lecithin		0

The data shows that the order of stability for the perfluorochemical oil phases is: Pf. tributylamine > Pf. hexane = Pf. methyldecalin > Pf. dimethylcyclohexane > Pf. methylcyclohexane > Pf. decalin. This is in agreement with the bulk stability data of table 4.3.2-1. The data for the effect of the emulsifier does not correlate with that of table 4.3.2-2. The bulk storage stability of Pluronic F-68 stabilized emulsions is greater than any of the other surfactants investigated, but in the forced coalescence stability test these emulsions are least stable. Thus the effect of the emulsifier cannot be accurately judged from the centrifugation studies on emulsions.

These centrifugation data did not comply to the mathematical treatment reported by Smith and Mitchell (1976).

4.3.3 The effect of additives

Oil in water emulsions of a given fluorocarbon chemical were prepared containing a small concentration of another oil phase (usually another fluorocarbon). The emulsions were allowed to age in medicine bottles stored at room temperature. Particle size distribution measurements were carried out at suitable time intervals.

4.3.3.1 Droplet size measurements

The particle size data and the formulae of the emulsions are given in table 4.3.3.1-1 and plotted in figure19 using the normalizing function D_t/D_o; where D_t is the mean droplet diameter at storage time, t, and D_o is the initial droplet diameter.

The addition of a small amount of perfluorotributylamine and perfluorodecalin into n-hexane enhanced the emulsion stability. The effect was more marked in the case of perfluorotributylamine. The addition of perfluorohexane reduced the emulsion stability.

Similarly it was found that the emulsion stability of perfluorodecalin emulsions was enhanced by the addition of a small concentration of perfluorotributylamine (0.1 M) into perfluorodecalin.

The particle size distributions did not comply with any particular mathematical distribution function, (e.g. normal, log normal).

Figure 19 Stability of Emulsions:

The Effect of addition of a small amount of a fluorocarbon oil into hexane

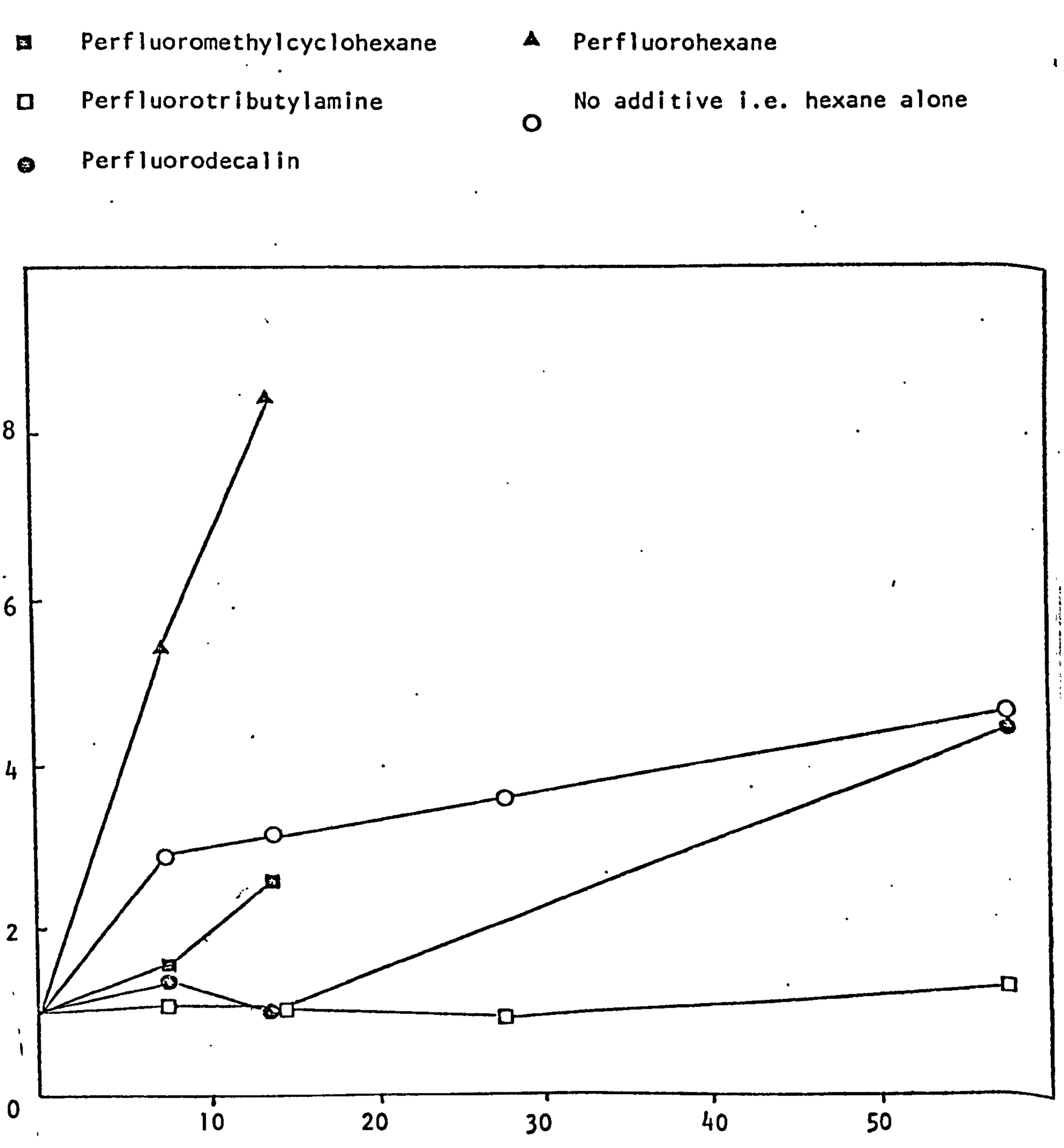


Table 4.3.3.1-1 Emulsion stability: the effect of additives

Oil (30% w/v)	Surfactant (4% w/v)	Diameter (µm)				
		Day 0	Day 7	Day 14	Day 27	Day 57
n-hexane	Sodium Dodecyl Sulphate	0.56	1.66	1.82	2.02	2.63
0.05 M Perfluorodecalin solution in n-hexane		0.24	0.33	0.24	-	1.1
0.05 M Perfluorotributylamine solution in n-hexane		0.33	0.37	0.33	0.30	0.42
0.1 M Perfluoromethylcyclohexane solution in n-hexane		0.31	0.49	0.84	-	-
0.1 M Perfluorohexane solution in n-hexane		0.53	2.86	4.46	-	-

4.3.4 The effect of employing an emulsifier system

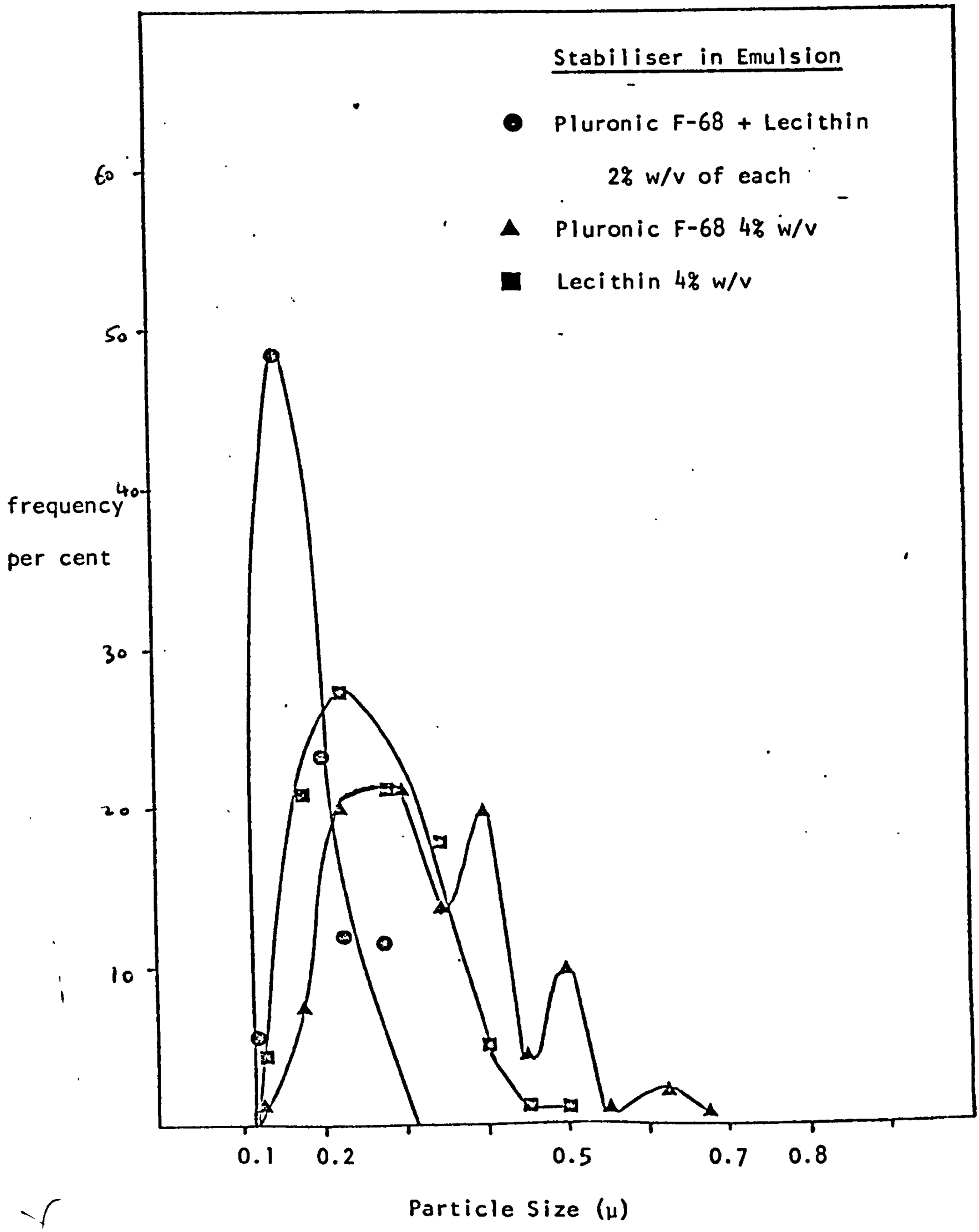
In an attempt to enhance emulsion stability on storage a combination of two emulsifiers was utilized. Certain combinations of surfactants were found to be incompatible, for example the use of Pluronic F-68 coupled with F.C. 170 destabilized the emulsion markedly compared with Pluronic F-68 alone. It was found that a precipitate formed when the solutions of the two surfactants were mixed. Thus the emulsifiers were precipitated out and consequently the emulsion was less stable. The particle size data of emulsions stabilized by a combination of two fluorinated surfactants (Forafac 1111 and F.C. 126) is given in table 4.3.2-1 and the mean particle size data of perfluorotributylamine emulsion, stabilized with a combination of lecithin and Pluronic F-68 is listed in table 4.3.2-2. Figure 20 illustrates the droplet size distributions of emulsions prepared using the emulsifier system (lecithin and Pluronic F-68) and using the two emulsifiers singly. It is apparent that the emulsifier system gives a narrower droplet size distribution and a smaller mean droplet diameter.

4.3.5 The problem of Fluoride (F^-) ions

The fluoride ion concentration was measured as described in subsection 3.3.1.1, using the fluoride electrode as a function of time of sonication at the full intensity of the sonic probe. The results are given in table 4.3.6-1. Two fluorocarbon oils were investigated in this experiment at a concentration of 10% w/v and the surfactant was Pluronic F-68 at a concentration of 4% w/v.

The results show that measurable concentrations of the fluoride

Figure 20 The Effect of using an emulsifier system on Particle Size distribution, oil phase : Perfluorotributylamine



ion are produced during sonication at full intensity of the sonic probe. Whether these concentrations are high enough to be fatal to animals remains to be investigated. It is also clear that the concentration of F^- ions produced is dependent on the fluorocarbon chemical.

4.3.5.1 Suppression of F^- ion production

It has been reported that sonication in an enclosed atmosphere of carbondioxide prevents the production of the fluoride ion on sonication of a perfluorocarbon chemical (Clark and co-workers, 1975). This was investigated further.

Table 4.3.5-1 lists the F^- ion concentrations after sonication in a carbondioxide atmosphere and a nitrogen atmosphere. The results show that carbondioxide inhibits the F^- ion production but nitrogen has no effect.

Table 4.3.5-1 Fluoride in production and its suppression during sonication

Oil phase 10% w/v in the emulsion and Pluronic F-68, 4% w/v	Sonication Time (Mins)	Concentration of Fluoride ions (Molarity)		
		Sonication		
		in Air	in CO_2	in N_2
Perfluoro tributylamine	5	1.6×10^{-4}	-	1.6×10^{-4}
	30	9.7×10^{-4}	2.1×10^{-6}	9.72×10^{-4}
	60	1.93×10^{-3}	4.6×10^{-6}	1.92×10^{-3}
	120	3.90×10^{-3}	1.05×10^{-5}	3.90×10^{-3}
Perfluoro-decalin	5	1.2×10^{-4}	-	1.21×10^{-4}
	30	8.0×10^{-4}	2.6×10^{-5}	8.0×10^{-4}
	60	1.44×10^{-3}	5.51×10^{-5}	1.43×10^{-3}
	120	2.86×10^{-3}	1.01×10^{-4}	2.80×10^{-3}
	180	4.31×10^{-3}	1.65×10^{-4}	4.30×10^{-3}

The data of table 4.3.5-1 has been plotted in figures 21 & 22. Figure 7 shows the calibration graph used to calculate the fluoride ion concentrations and table 4.3.5-2 lists the data used to plot the calibration graph.

Figure 21 showing fluoride ion production and its suppression by CO_2 during sonication

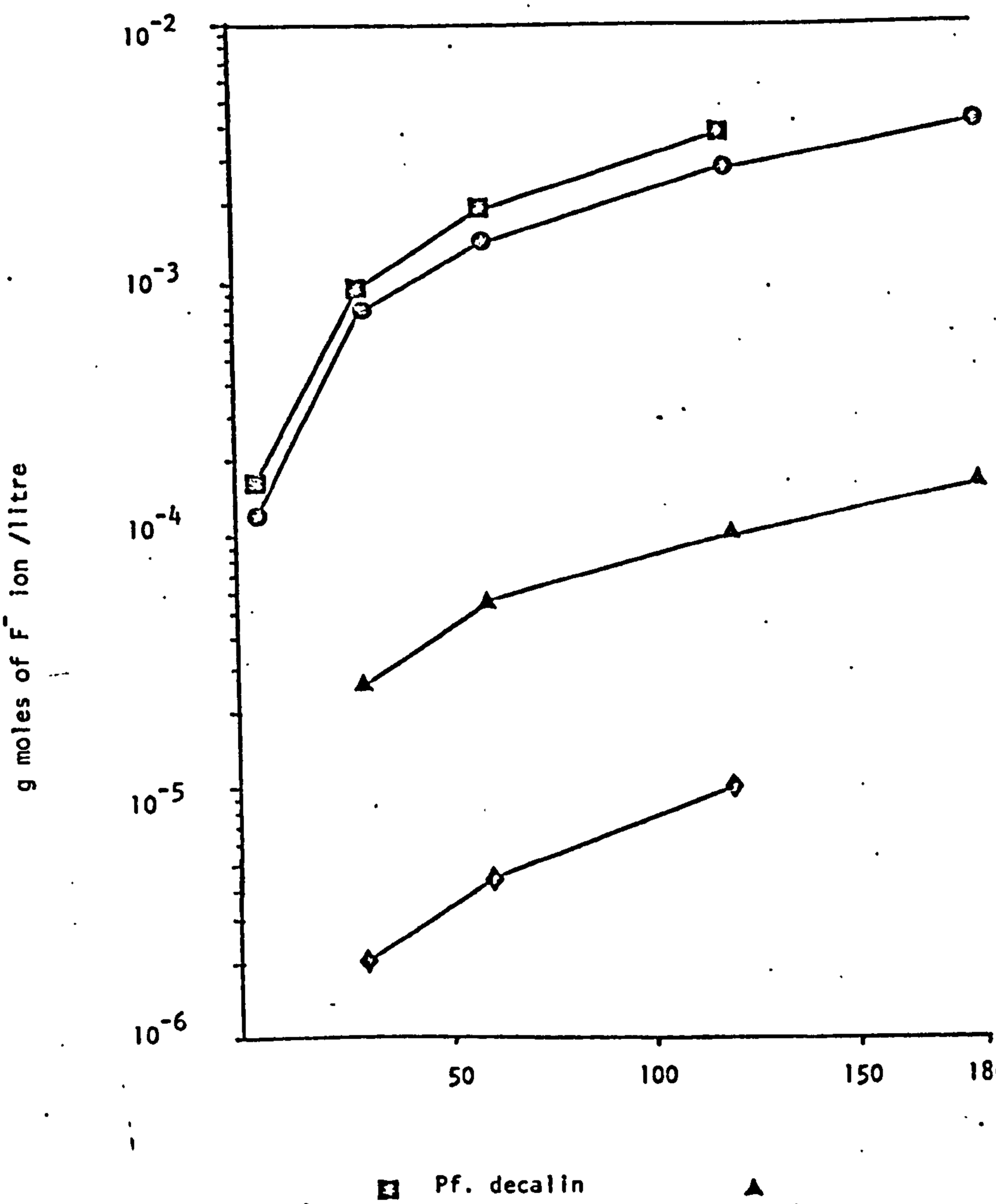


Figure 22 Suppression of Fluoride ion production by Carbon-dioxide

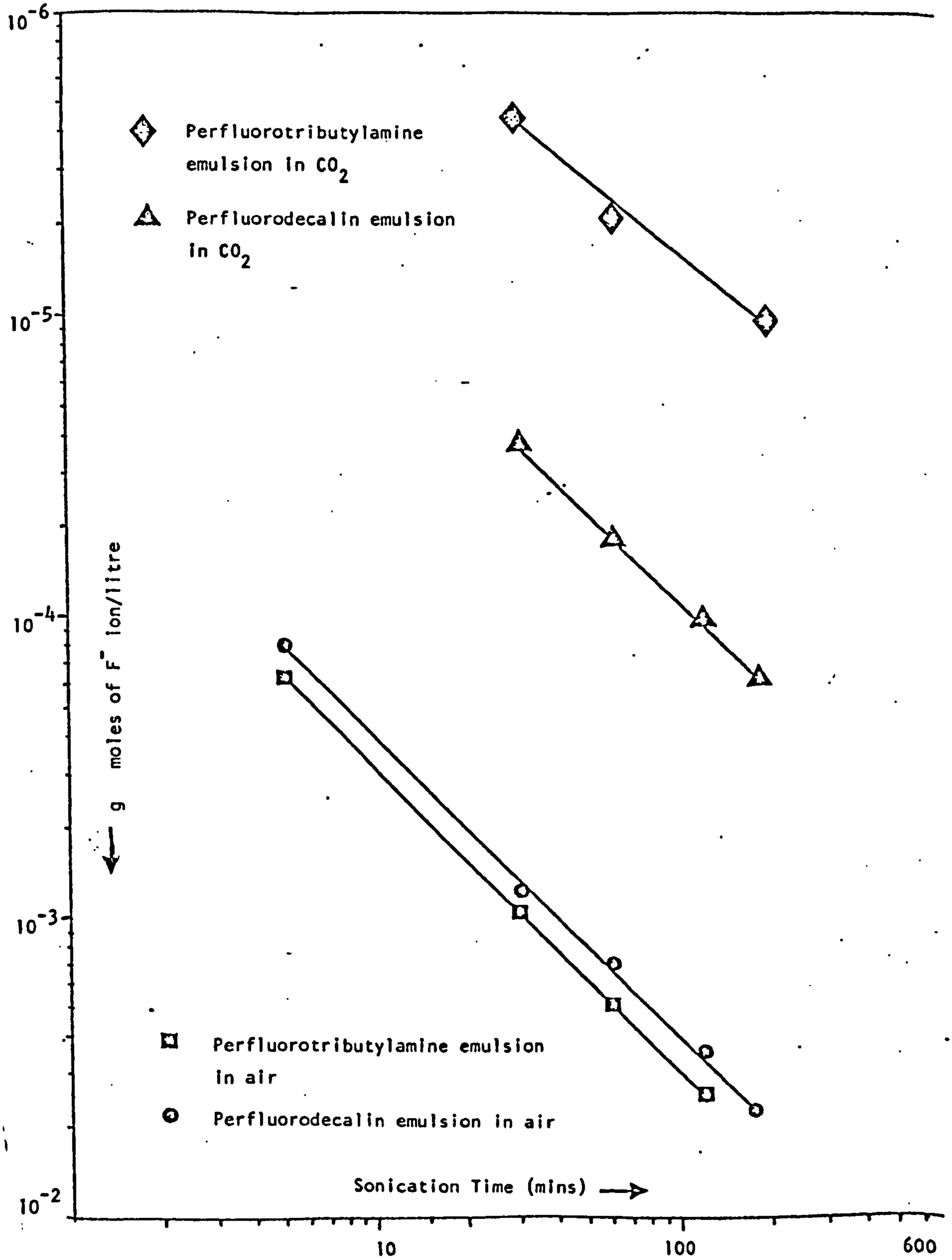


Table 4.3.5-2 Fluoride in concentration: calibration graph data

E	E_o	$_pF$
118.7	52.9	1.1
176.2	55.2	2.0
235.4	57.2	3.0
294.6	58.2	4.0

Note that $E = E_o + 2.303 \frac{RT}{F} {}_pF$ See subsection 3.3.1.2.1

4.3.6 Sterilization of fluorocarbon emulsions

Two methods of sterilization were investigated, namely, steam sterilization (autoclaving) and filtration through a milipore filter under aseptic conditions. The two methods were also used in combination to determine if there were any advantages, over when these were used separately, such as autoclaving at a lower temperature.

4.3.6.1 Autoclaving

The autoclaving temperature was 126°C for 15 minutes. The emulsions were allowed to cool by standing at room temperature. The heating up time was 10 minutes. The sterility tests showed that the emulsions were sterilized by exposure to the above conditions. The mean particle sizes of the sterilized and the same non-sterile emulsions is given in table 4.3.6.1-1.

Table 4.3.6.1-1 Emulsion stability: the effect of autoclaving on mean droplet diameter

Surfactant 4% w/v	Oil: Perfluorotributylamine 20% w/v					
	Mean droplet diameter (μm)					
	Day 0		Day 14		Day 35	
	Sterile	Non-Sterile	Sterile	Non-Sterile	Sterile	Non-Sterile
SDDS	0.49	0.24	0.96	0.59	1.1	1.2
Pluronic F-68	0.39	0.38	0.41	0.41	0.45	0.44
Lecithin	0.20	0.21	0.39	0.41	0.43	0.88
Pluronic + lecithin	0.19	0.19	0.22	0.23	0.27	0.30

The results indicate that the effect of autoclaving on emulsion stability is dependent on the emulsifier for a given oil phase. With the exception of SDDS, the effect of autoclaving on the initial droplet diameter was negligible. The ageing behaviour of emulsions, prepared using Pluronic F-68 and the emulsifier system (Pluronic F-68 combined with lecithin), that had been sterilized and those that were non-sterile, was almost identical. With SDDS as the surfactant the sterile emulsion was slightly more stable on storage; this may be the result of a small amount of the surfactant being hydrolysed to the alcohol which would act as a co-surfactant forming a closely packed film and thus enhancing the emulsion stability.

The sterilized emulsion with lecithin as the stabilizer was also more stable on storage than the non-sterile one. This can be attributed to the breakdown of lecithin by micro-organisms in the non-sterile preparation, and the absence of any breakdown

of lecithin in the sterile emulsion. After about 8 weeks storage the growth of micro-organisms in the lecithin stabilized emulsions was visible to the unaided eye.

4.3.6.2 Sterilization by filtration

A Swynex filtration unit incorporating a milipore membrane filter of 0.45 μm pore size was utilized for this experiment. An emulsion of perfluorotributylamine stabilized by Pluronic F-68 was sterilized by this method. The procedure was carried out in the sterile air cabinet.

The sterility tests showed that the emulsion had been sterilized after the filtration procedure. Particle size analysis showed that the mean droplet diameter decreased from 0.41 μm to 0.30 μm after the filtration. Quantitative analysis for the fluorocarbon by GLC showed that 20% of the fluorocarbon oil phase had been lost during the procedure.

4.3.6.3. Filtration and autoclaving combined

The aqueous phase and the oil phase were filtered through a bacteria proof filter (milipore 0.45 μm pore size) and the emulsion prepared using autoclaved sonic-probe tip. The emulsion was autoclaved at a reduced temperature and exposure time. The sterility tests showed that the product was sterilized. Particle size analysis showed that this sterilization procedure had the minimal effect on the initial mean particle size and the ageing behaviour of the emulsion was not altered. Therefore this method is recommended.

4.3.7 Storage temperature

Emulsions of Perfluorotributylamine were stored at 4°C, room temperature and 50°C and particle size distributions determined at various time intervals to estimate stability. The effect of temperature cycling on emulsion stability was also investigated. Temperature cycling was carried out by freezing the emulsion in liquid nitrogen and then allowing it to thaw at room temperature. The mean droplet diameter was measured after each temperature cycle. Table 4.3.7-1 lists the data for the effect of storage temperature on the mean droplet diameter.

Table 4.3.7-2 lists the results obtained from the temperature cycling experiments.

Table 4.3.7-1 Emulsion stability: the effect of storage temperature

Storage Temperature °C.	Oil phase: Perfluorotributylamine 10% w/v Surfactant: Pluronic F-68 4% w/v		
	Mean Droplet diameter (μm)		
	Day 5	Day 35	Day 60
4°	0.39	0.41	0.43
23°	0.40	0.44	0.6
50°	0.40	0.49	0.7

Table 4.3.7-2 Emulsion stability: the effect of freeze-thaw cycles

Surfactant 4% w/v	Oil Phase 10% w/v	Mean Droplet Diameter (μm)			
		Cycle 0	Cycle 1	Cycle 2	Cyle 3
Pluronic F-68	Pf. tributylamine	0.41	0.5	0.7	1.1
	Pf. methylcyclohexane	0.40	0.6	0.9	1.5
	Pf. decalin	0.40	0.6	0.95	1.8
SDDS	Pf. tributylamine	0.45	1.62	1.94	2.5
	Pf. decalin	0.48	2.0	oil separated	
lecithin	Pf. tributylamine	0.30	0.46	0.8	1.5
	Pf. methylcyclohexane	0.40	0.73	1.0	2.0
	Pf. decalin	0.23	0.6	0.98	2.2.

These data have also been plotted, (figures 23 and 24) using the normalizing function D_t/D_o where D_t is mean droplet diameter at time, t, (or D_n, where n signifies freeze thaw cycle number) and D_o is initial diameter. It is clear from these data that for optimum stability the fluorochemical emulsions should be stored at 4^oC or less but must not be allowed to freeze.

The order of stability for the oil phases is: Pf. tributylamine > Pf. methylcyclohexane > Pf. decalin. The emulsions stabilized by Pluronic F-68 were the most stable against freeze thaw cycles where as those stabilized by SDDS were least stable, lecithin was intermediate.

These results correlate quite well with the bulk stability of these emulsions on storage at room temperature. Therefore it may be concluded that an estimate of emulsion stability could be obtained by subjecting the emulsions to the stress of freeze thaw cycles.

4.4 Oxygen release and solubility in fluorocarbon emulsions

The potentiometric recorder used (MSE Ltd., Electro-plus) had a scale for directly reading the percentage of dissolved oxygen.

The percentage of dissolved oxygen in water was found to be 3%. This did not vary significantly if a surfactant was dissolved in the water at the same concentration as in the aqueous phase of the emulsion under test. The percentage of oxygen dissolved in fluorocarbon emulsions saturated with oxygen by bubbling the

Figure 23 Fluorocarbon Emulsion Stability:

The Effect of Storage temperature on mean

droplet diameter

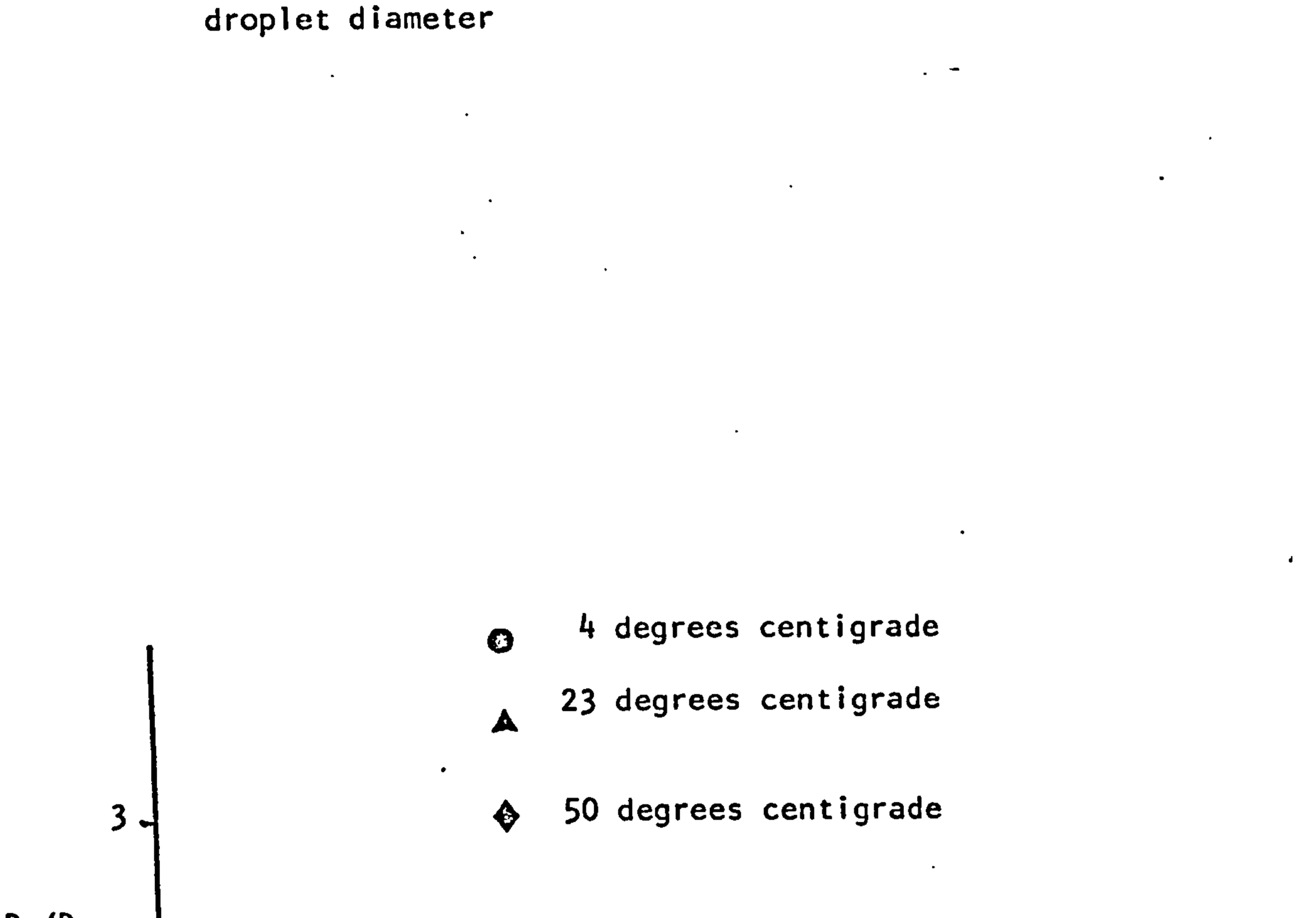
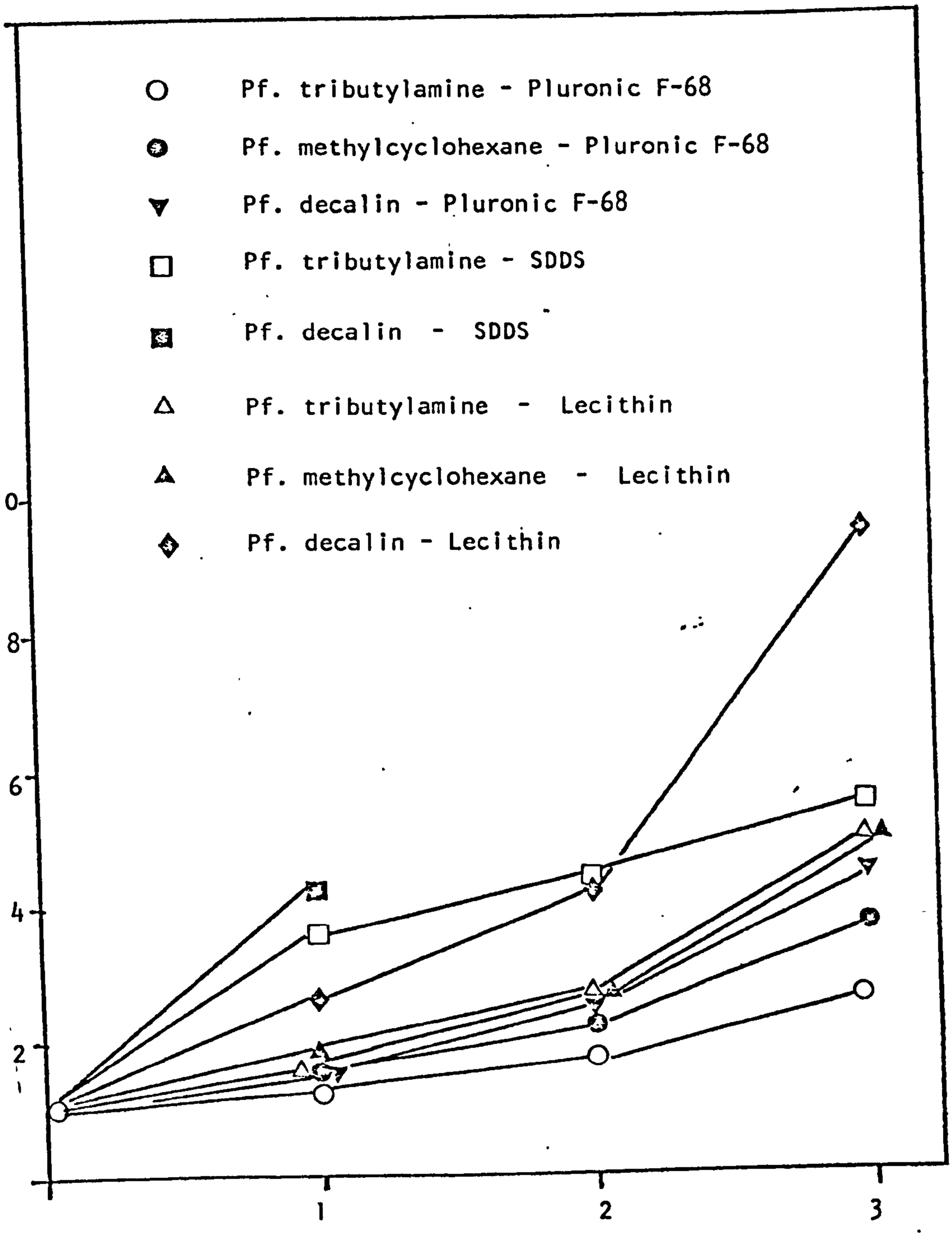


Figure 24 Fluorocarbon Emulsion Stability: The Effect of Freeze-thaw cycles on the mean droplet diameter.



oxygen through them in a sealed atmosphere was found to be between 40.8 to 41% v/v. The effect of the oil phase on the percentage of oxygen dissolved in fluorocarbon emulsions was found to be negligible in the oils studied.

It was difficult to follow the oxygen release characteristics of fluorocarbon emulsions because the oxygen release was almost instantaneous, reaching equilibrium value within a second. Therefore the data given in table 4.4-1 represents only approximate readings. These data have also been illustrated graphically in figure 25.

Table 4.4-1 Oxygen release from fluorocarbon emulsions

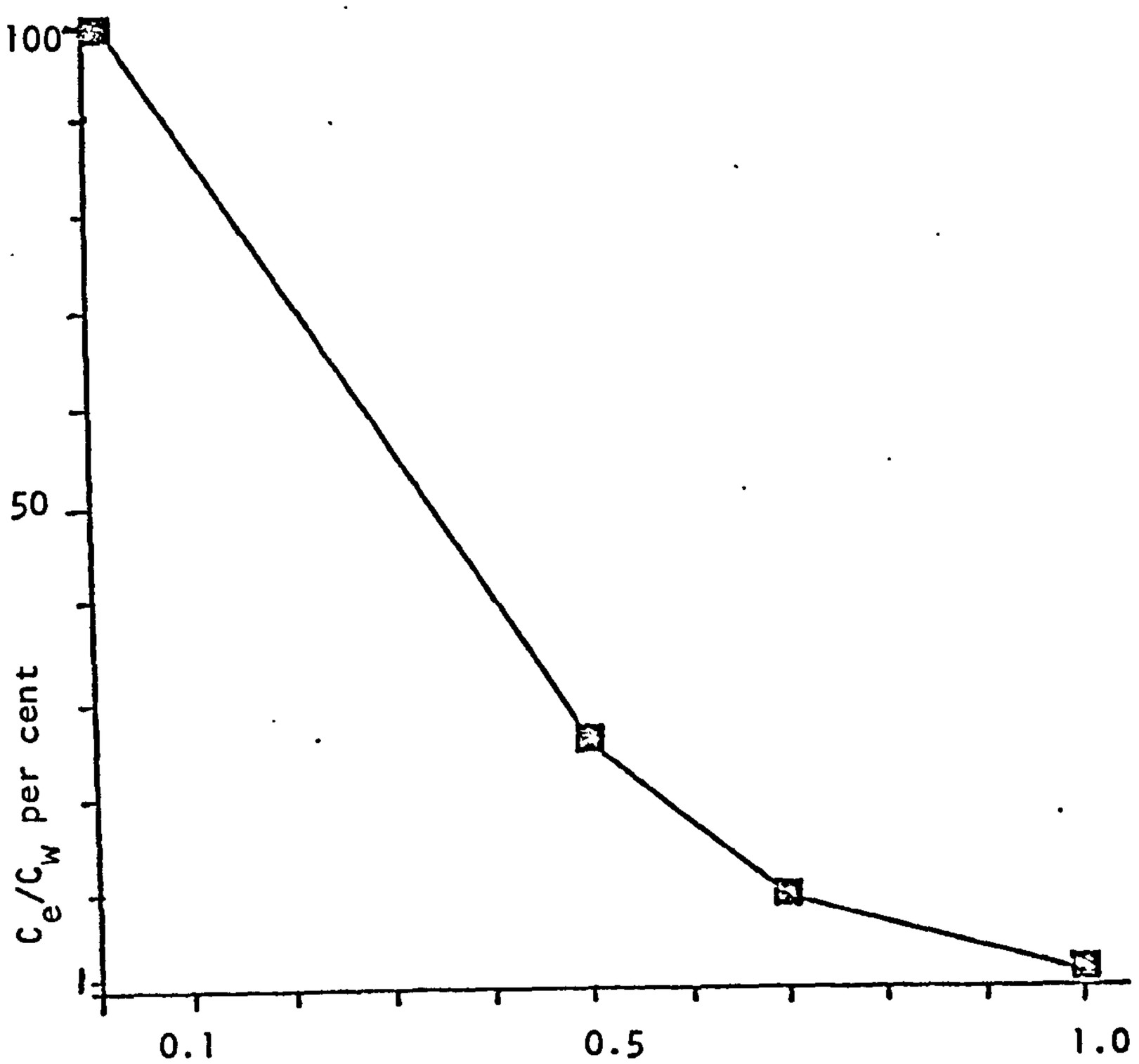
Time (Sec)	C_e/C_w (%)	
	Perfluorodecalin emulsion	Perfluorotributyl-amine emulsion
0	100	100
0.5	26.1	25.6
0.7	10.1	10.2
1.0	1.0	1.0

Note: C_w is saturated concentration of oxygen in water and C_e is concentration of oxygen in emulsion. The emulsions contained 10% w/v of the fluorocarbon oil phase and Pluronic F-68 4% w/v as the emulsifier.

4.5 Phagocytosis of fluorocarbon emulsions

First a calibration graph was prepared using latex particles of

Figure 25 Oxygen release from fluorocarbon Emulsions



C_e is concentration of oxygen in emulsion

C_w is concentration of oxygen in surfactant solution saturated with oxygen.

narrow particle size distribution and having a mean particle size of 1.5 μm (Coulter Electronics). Small accurately measured aliquats of the latex suspension were added to 10 ml of the buffer solution and the absorbance measured at 504 nm wavelength (456 nm wavelength could also be used).

The data is given in table 4.5-1 and plotted in figure 26 .

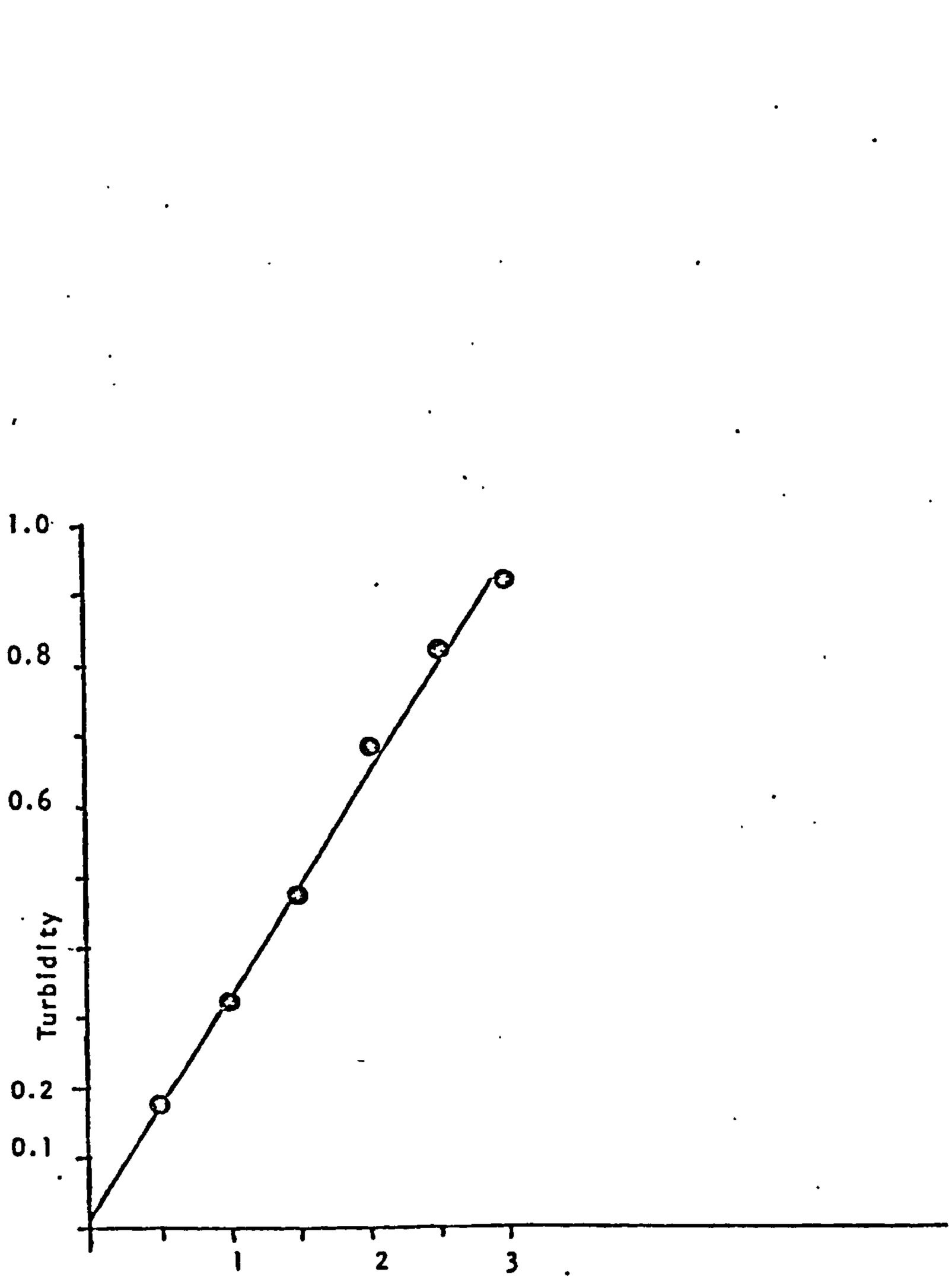
Table 4.5-1 Phagocytosis of fluorocarbon emulsions: calibration graph data

Volume of latex in 10 ml. of buffer	Absorbance (turbidity) at 504 nm
0.5 ml	0.18
1.0 ml	0.32
1.5 ml	0.48
2.0 ml	0.69
2.5 ml	0.83
3.0 ml	0.92

It can be seen that there is a direct relationship between absorbance and the volume of latex suspension in 10 ml of buffer. Since a certain volume of latex suspension will have a fixed number of particles we can assume that the number of particles in suspension is directly related to turbidity. Therefore measurement of turbidity at various times could be used to follow the rate of phagocytosis of emulsion particles.

The data for the phagocytosis of emulsions is listed in table 4.5.-2

Figure 2 6 Phagocytosis of Fluorocarbon Emulsions : Calibration graph showing relationship between turbidity and particle number in latex suspension



and plotted in figure 27. Typical absorbance trace is shown in figure 28.

Table 4.5-2 Phagocytosis of fluorocarbon emulsions, in vitro.

Oil phase: Perfluorotributylamine 10% w/v

Pluronic F-68		Lecithin		SDDS	
Time (Mins)	Turbidity at 504 nm	Time (Mins)	Turbidity at 504 nm	Time (Mins)	Turbidity at 504 nm
0	0.9	0	0.9	0	0.9
10	0.89	20	0.55		
30	0.83	40	0.30	10	0.68
50	0.75				
90	0.63	70	0.15		
120	0.55	90	0.10		
150	0.5				

Note that after 10 minutes, due to the toxicity of the SDDS stabilized emulsion the Phagocytes were destroyed and therefore the experiment could not be followed any further.

The results show that Pluronic F-68 stabilized emulsion was cleared much slower than the lecithin or SDDS stabilized emulsions. It was also found that for a given emulsion system the emulsion having a smaller droplet diameter on average, was removed at a slower rate than the same emulsion having a larger mean droplet diameter.

Figure 27 Phagocytosis of Fluorocarbon Emulsions: Effect of the Emullsifier.

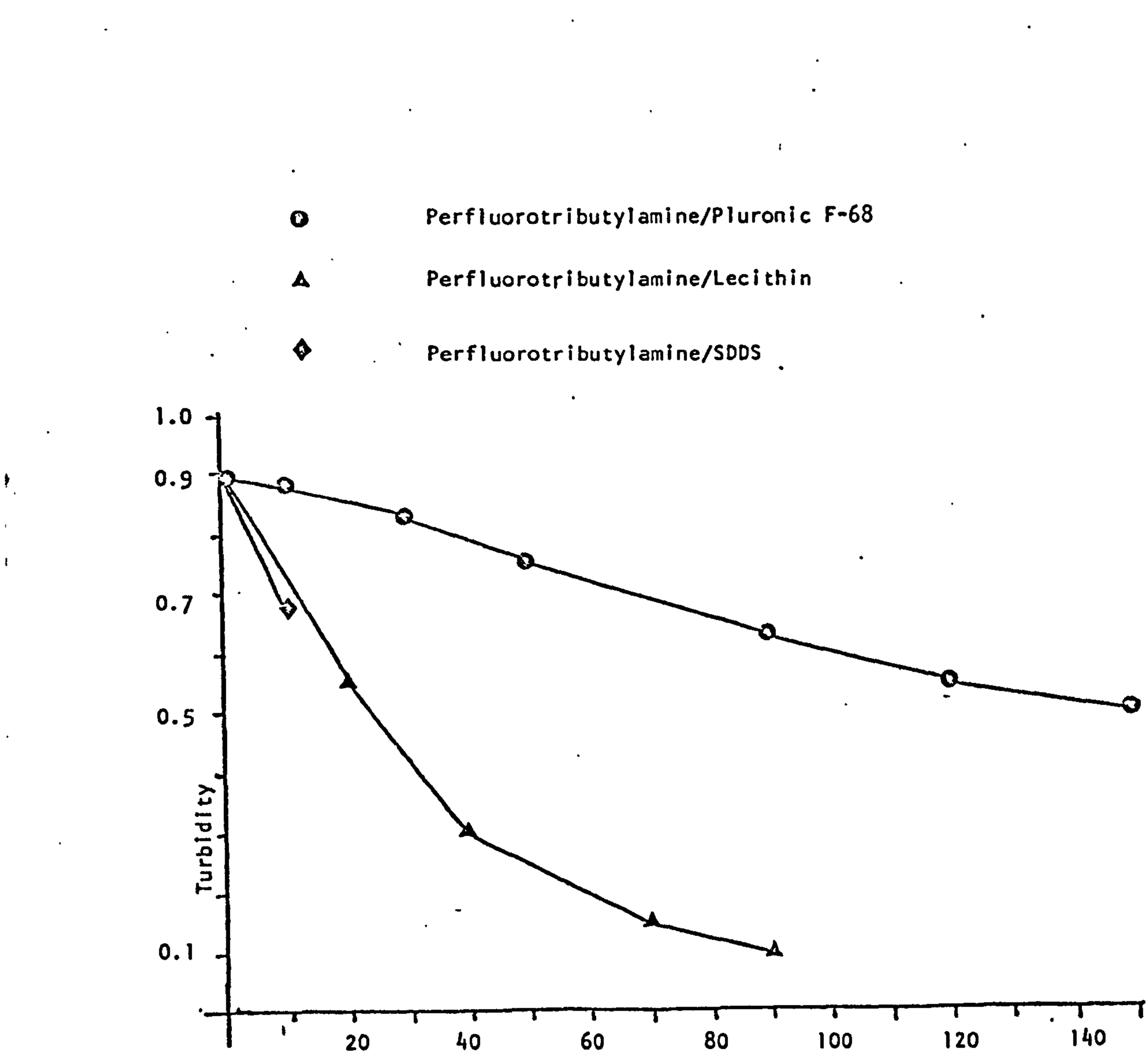
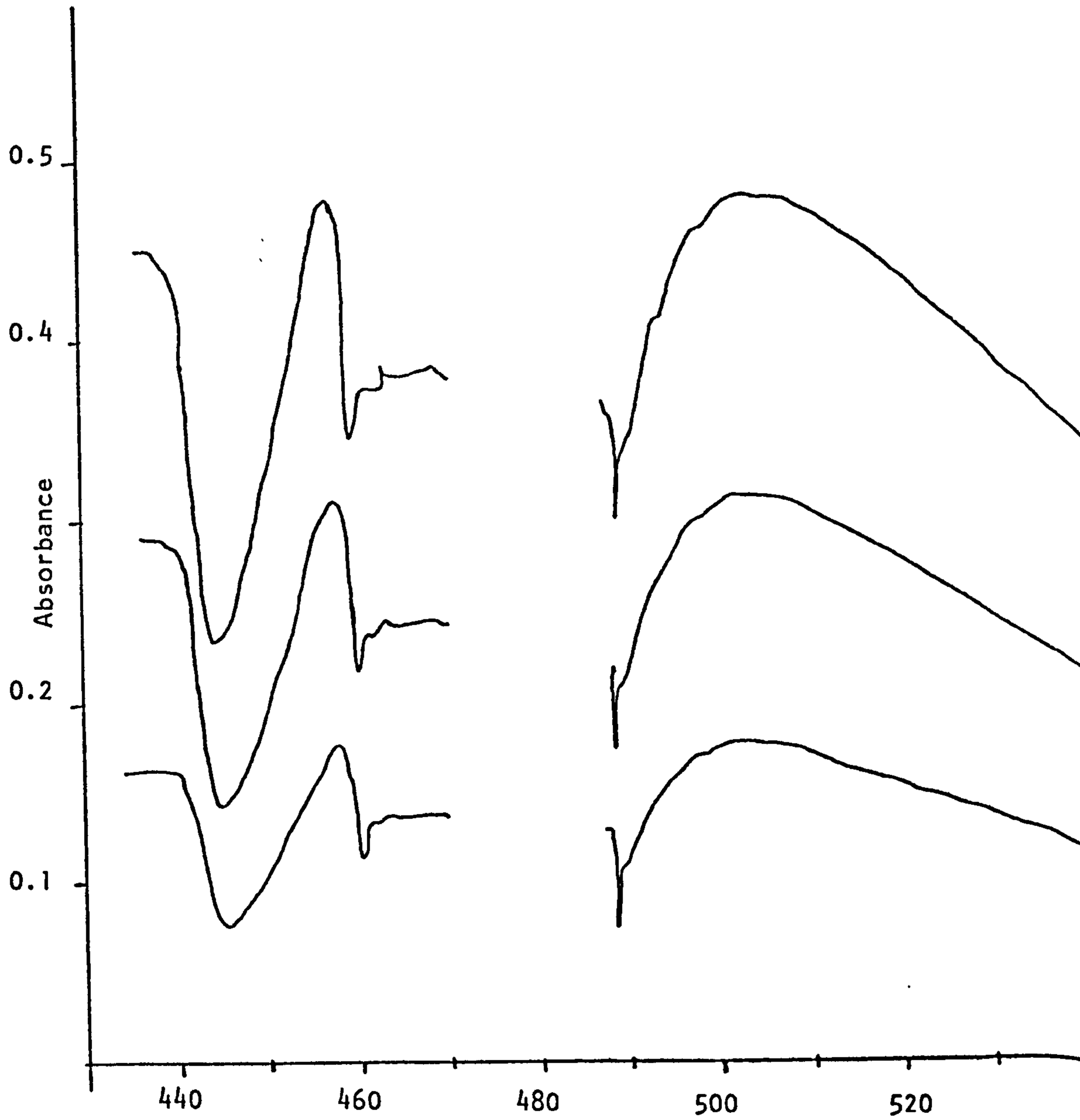


Figure 28. Typical Absorbance Traces obtained in the Phagocytosis experiments.



5. DISCUSSION

5.1 Surfactant adsorption

5.1.1 Interfacial Tension

It is well established that the interfacial tension between two liquids depends upon the chemical nature, interaction and orientation of molecules at the interface. The interfacial tension is sensitive to the presence of small concentrations of impurities or added surfactants. Many attempts have been reported to interpret interfacial tension in terms of intermolecular forces within and between two fluid phases, (Antonow, 1907; Girifalco & Good, 1957; Fowkes, 1963 & 1964). More recently the work of Zografi & Yalkowsky (1974) has related the differences in the interaction at alkane-water interfaces to the number of dispersion interactions per unit area of the interface. It was found that γ_w^d (the dispersion interactions contribution to the surface tension of water) could be explained by the different group densities obtained with the various alkanes. However, the theory of molecular interaction at the interface is still largely undeveloped (Aveyard & Haydon, 1973; Zografi & Yalkowsky, 1974). Despite this, the extent of adsorption of a surface active agent can be estimated from interfacial tension data. Various parameters can be used to compare different systems. The area per adsorbed surfactant molecule at the interface can be calculated from a plot of interfacial tension vs. log concentration and how it varies with different oils can be useful for comparing different oil phases. Similarly the extent of adsorption can be compared for different oils by plotting π (surface pressure) against surfactant concentration. The change in surface pressure (π)

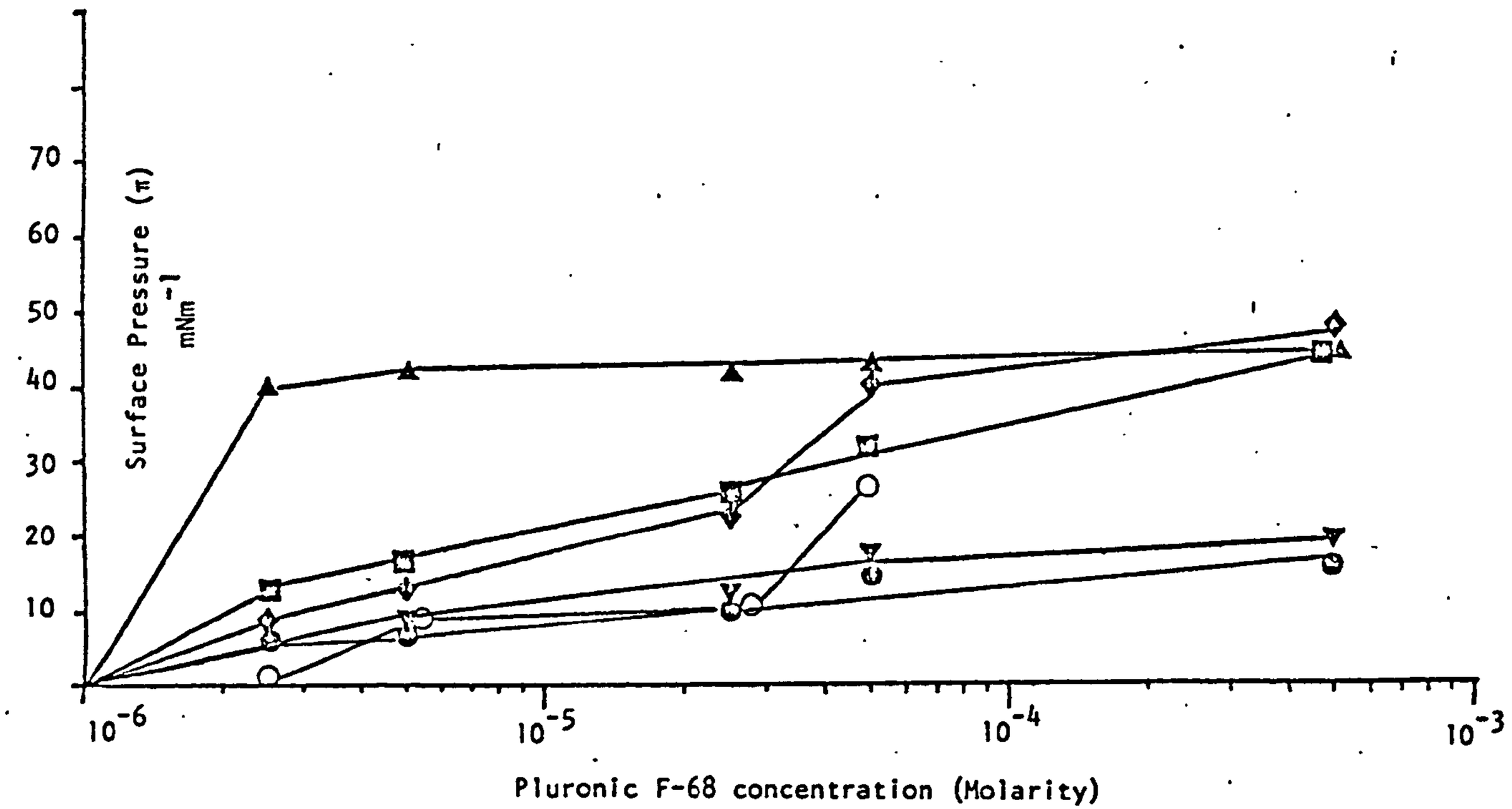
with surfactant concentration for fluorocarbon oil phases is shown in Fig.31 using Pluronic F-68 as the surfactant. It is clear that the extent of surfactant adsorption is very much dependent on the oil phase. Similarly if we compare the areas per molecule at a given surfactant concentration we find it varies with the oil phase. Generally, many surfactants are poorly adsorbed at the perfluorochemical/surfactant solution interface.

For example, at a concentration of 1×10^{-3} mol dm^{-3} of SDDS in water, the areas occupied by each surfactant molecule in the case of fluorocarbon oils is greater than in the case of hydrocarbon oils, see table 5.1-1. The data quoted for hydrocarbon oil phases is that obtained by Smith (1975).

Table 5.1-1 Surfactant adsorption

Oil Phase	Area per molecule (nm^2) surfactant : SDDS 10^{-3}M
Perfluorotributylamine	10.7
Perfluorodecalin	13.7
Perfluoromethyldecalin	19.3
Perfluoromethylcyclohexane	21.0
Perfluorohexane	9.2
Hexane	0.85
Dodecane	1.022
Hexadecane	0.984
Toluene	1.057

Figure 31 Adsorption of Surfactant at the Fluorochemical - Pluronic F-68 Solution Interface



When trying to correlate interfacial tension data with bulk emulsion stability it should be remembered that the interfaces involved in interfacial tension measurements are usually much less curved than the interfaces present in emulsions. Excluding gravitational effects the pressure difference across a curved interface increases with increased curvature.

The importance of interfacial tension and surfactant adsorption in emulsion formulation has been highlighted by spontaneous emulsification studies and formation of microemulsions. In the case of microemulsions the question is: are these microemulsion droplets merely swollen micelles? The key to the problem centres on the fundamental question of thermodynamic stability. Gerbacia and Rosano (1973) have reported on the dynamic role played by the co-surfactant in the formation of microemulsions. They placed a 50 ml sample of n-hexadecane on top of 50 ml of 1.37×10^{-3} molar aqueous sodium dodecyl sulphate solution and injected pentanol into the oil phase while monitoring the interfacial tension continuously using an automated Wilhelmy plate. They found that for a period of about one minute the interfacial tension fell transiently to near zero, due to transfer of alcohol across the interface, but then rose back again to about its original value. During the period when interfacial tension was about zero, the free energy of emulsion formation is less than zero, and spontaneous emulsification is possible but as interfacial tension rises the free energy of emulsion formation becomes greater than zero and spontaneous emulsification ceases. Thus, microemulsion formed in this case, in its final form, is not thermodynamically stable. However, it could be

kinetically stable, due to the presence of an energy barrier preventing breakdown. Therefore microemulsions, of this type, are different from the thermodynamically stable swollen micellar systems.

5.1.2 Nature of the Adsorbed Film

The studies of Glass, Lundberg & Bailey (1970) have confirmed that the stability of droplets depends on the resistance of the interfacial film to rupture. The resistance of the film will depend on the type of adsorbed film at the interface which in turn is dependent on the nature of the surfactant and the oil phase. For a given surfactant and oil phase system the type of film formed is dependent on the surfactant concentration. If the concentration is insufficient to cover the interfacial area completely a gaseous or expanded liquid film may result.

The interfacial tension data shows that surfactants are poorly adsorbed at the perfluorochemical-water interface. This is because the adhesive forces between the surfactant and oil molecules are poor compared with hydrocarbon oils (see table 5.1-1). The limiting areas at the oil-water interfaces are higher than at the air-water interfaces. It is generally agreed that this is caused by reduced cohesive forces between the surfactant molecules.

In the fluorocarbon emulsion systems it is likely that an expanded or even gaseous type of film was present because upon emulsification the interfacial area was enormously increased and the surfactant concentration employed (e.g. Pluronic F-68 5×10^{-3}M) was probably insufficient to completely cover the interfacial area.

The surface pressure (π) and the area per adsorbed surfactant molecule data of table 4.1-2 show marked deviations from the "ideal" gaseous state. This was probably caused by significant ageing effects during the measurements of interfacial tensions. The deviation is more in the case of lecithin and SDDS, no doubt due to the repulsion between ionic heads of the surfactant molecules in addition to the ageing effects.

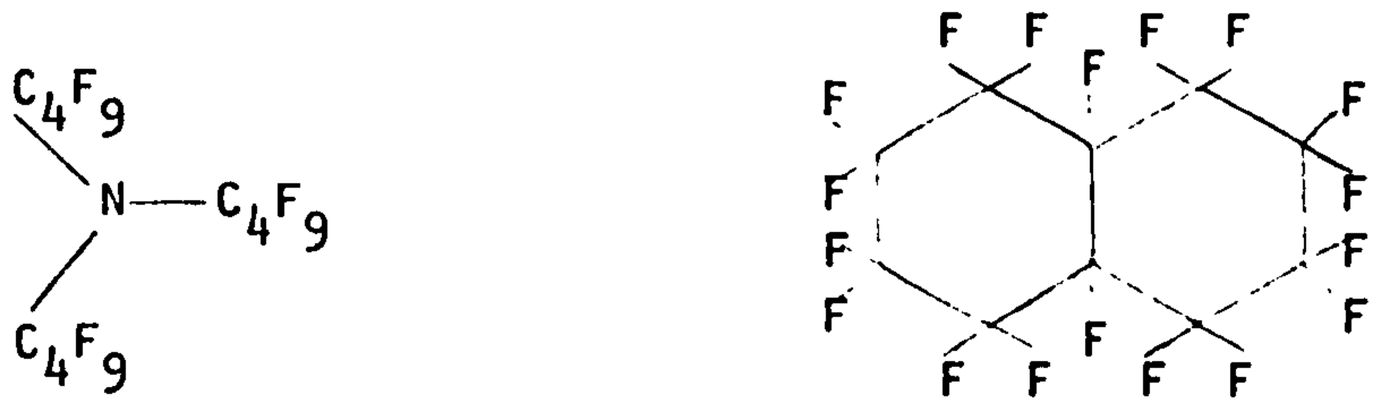
Out of the perfluorochemicals studied, perfluorotributylamine interfaces showed better adsorption. This was attributed to the more branched chemical structure of the molecule and the presence of the hetero atom in the molecule. Perfluorohexane also showed better adsorption, again it was a chain like structure as opposed to the cyclic structure of perfluorodecalin and perfluoromethyl-, and dimethyl-, cyclohexanes.

5.1-3 The Effect of the Oil Phase

The area per molecule data of table 4.1-2 illustrate that the nature of the oil phase can affect the amount of surfactant adsorbed, for example, the area per molecule adsorbed at Perfluoromethyldecalin/Pluronic F-68 solution interface is greater than the Perfluorotributylamine - Pluronic F-68 solution interface for a given concentration of Pluronic F-68. This indicates that there is greater adsorption at the Perfluorotributylamine interface. The area per molecule data of table 4.1-2 shows some anomalies, e.g. initially the area per molecule decreases with increased surfactant concentration which is to be expected as a more closely packed film is produced, but after a certain value it starts to increase with increasing

surfactant concentration. It is thought that this is caused by association of surfactant molecules to form a larger complex structure having a larger area. Ageing effects at the interface during tension measurements also cause errors. Generally, the area per adsorbed molecule for a given surfactant at a given concentration is larger in the case of fluorocarbon oils than the corresponding hydrocarbon oils. This is not surprising because the free energy change for the transfer of a $-CH_2-$ group of the surfactant chain from aqueous phase to a fluorocarbon oil is likely to be less than transfer to a hydrocarbon oil because the hydrocarbon chain of the surfactant is likely to interact more with a hydrocarbon oil than a fluorocarbon oil.

The question arises, why is there a difference between adsorption by different fluorocarbon oils, since they have similar physical characteristics? The answer lies in the differences in polarity of the compounds. Consider, for example, the chemical structures of Perfluorotributylamine and Perfluorodecalin (diagram below).



Perfluorotributylamine Perfluorodecalin

Diagram showing chemical structure of two perfluorochemicals.

The Electron distribution and therefore the polarity of these compounds is likely to be quite different. The polarity differences may be estimated from the measurements of dipole moments or dielectric constants.

Furthermore, the chain structure of perfluorotributylamine means that it can probably interact to a greater extent with surfactant molecule chains than perfluorodecalin. This would mean that the emulsions of perfluorotributylamine would be more stable for a given surfactant. This, indeed, was found to be the case.

5.2 Single Droplet Stability

In all single droplet coalescence experiments a distribution of rest-times was obtained. The scatter of the measured droplet rest-times increased with overall stability. Some reproducibility was obtained by thorough cleaning of the apparatus and minimising vibration. Many other reasons have also been proposed for the scatter of rest-times.

Cockbain & McRoberts (1953) attributed it to surfactant displacement. Biswas & Haydon (1962) and Lee & Hodgson (1968) thought it was due to inhomogeneity of the adsorbed film. Gillespie & Rideal (1956) found it was also due to thermal effects. They also postulated that the rupture of the draining film could occur at different thicknesses thus giving a distribution of rest-times. Therefore the minimum rest-time would correspond to the film thickness at which rupture can occur and for droplets persisting longer coalescence can occur at any time when a disturbance sufficient to rupture the film occurs. Brown & Hanson

(1966) have also argued that the angle of approach to the interface will vary between droplets which have travelled some distance giving rise to differences in the thickness of the trapped film and hence the drainage time. The majority of reports have indicated that 50 to 100 measurements are sufficient for a statistical analysis to derive reproducible stability parameters.

5.2.1 Mechanism of Coalescence

The coalescence process of a single drop at a plane interface may be divided into the following consecutive stages:

(1) Arrival of the Droplet.

The droplet rises or falls, depending on its density, through a liquid medium (the continuous phase).

It can be assumed that the droplets will reach the interface with a constant terminal velocity since the droplet size would be constant. However, there will be a slight variation between the droplets of different oils, under identical conditions, because of density differences between the oils.

(2) Impact.

The droplet hits the interface, with sufficient force to make the droplet "bounce" thus causing ripples in the interface. This results in entrapment of a film of the continuous phase between the droplet and the plane interface.

(3) Film Drainage.

Gravity exerts a pressure on the trapped film, causing the continuous phase to drain radially from between the droplet and the interface. This may result in

deformation of droplet and/or the interface.

(4) Rupture of the Film.

Eventually the film becomes so thin that it ruptures and the droplet coalesces with its bulk phase. Obviously the thickness of the draining film will affect the probability of film rupture and therefore the droplet rest-time.

The measured droplet life-time at the plane interface is, therefore, a composite of all these processes and is dependent on any interfacial film adsorbed at the two oil-water interfaces, which is dependent on the nature of the surfactant and the oil phase.

5.2.2 Effect of the Surfactant

The coalescence of the fluorochemical oil phase droplets was instantaneous in the absence of a surfactant. Addition of a surfactant enhanced the droplet stability at the plane interface. This may be the result of changes in the rate of film drainage or the resistance of the film to rupture or both.

A number of authors have postulated that the major effect of a surfactant is to slow the rate of film drainage (Gillespie & Rideal, 1956; Hodgson & Lee, 1969; Hodgson & Woods, 1969). Gillespie & Rideal (1956) have suggested that the mechanism of film rupture is likely to be the same with or without the surfactant. Lee & Hodgson (1968) have proposed that when the draining film thickness is below 100 nm Van der Waals attraction occurs and random molecular motions (at thicknesses below 10 nm) cause rapid thinning and rupture of the film.

The factors affecting film drainage are complex. The viscosity of the interface would be affected by the surfactant and a viscous or viscoelastic film should retard film drainage. The solvation envelope of water around surfactant molecules at the interface may provide an additional barrier to film drainage. Hodgson et al (1968 and 1969), Hartland (1970) and Burill & Woods (1973) have demonstrated that even small concentrations of a surfactant alter the film drainage rate and increase the stability. A surfactant film around a droplet may also inhibit internal circulation leading to slower drainage. In the case of charged interfacial film, additional stability may be provided by electrical factors such as electric double layer repulsion. The decrease in interfacial tension due to a surfactant may allow greater deformation of the droplet and interface leading to an increase in the film area which has to drain and therefore drainage takes longer before film rupture can occur.

An alternative view expressed by Cockbain & McRoberts (1953) is that the stabilising effect of the surfactant is due to increased resistance of the film to rupture and its reduced wettability by the dispersed phase. Nielsen (1958) made a similar suggestion and Watanabe & Kusui (1958) thought that the droplet stability was determined by the adsorbed film characteristics. They considered the drainage time to be negligible and proposed that the coalescence mechanism was that a defect formed in the film in the region of droplet-interface contact, probably due to surfactant desorption. Biswas & Haydon in 1962 reported that rapid drainage occurred from aqueous lamellae even in the presence of viscous adsorbed films and that the stability was dependent on

the ability of the film to withstand the droplet weight. The studies of El-Shimi & Izmailova (1966) and Glass, Lundberg & Bailey (1970) have confirmed that the stability depends on the resistance of the film to rupture.

5.2.3 The Effect of the Oil Phase

A number of fluorocarbon oil phases were investigated including the solutions of perfluorohexane in n-hexane. The results indicate a clear dependence of the droplet rest-times, at the plane interface, on the nature of the oil phase. Table 5.2.3-1 lists the relative viscosity, the density difference between the two phases ($\Delta\rho$), for the different oils. It was considered by Jeffreys & Davies (1971) that these physical properties of the oil phase affect the film drainage rate and hence the droplet life-times at the plane interface.

The effect of the oil phase viscosity on film drainage and coalescence processes has been reported by a number of workers, (Ishida, Sonada & Yoshida, 1968; Charles & Mason, 1960; Burill & Woods, 1973). Jeffreys & Davies (1971) have concluded that the phase viscosity ratio will affect stability and that increased viscosity of continuous phase relative to the droplet phase will reduce film drainage rate, whilst a high oil viscosity (the droplet phase) may retard film rupture. The data of table 5.2.3-1 indicate that no such correlation exists in the case of the systems studied.

The relationship between density difference ($\Delta\rho$) and droplet stability is complex. An increase in $\Delta\rho$ means greater buoyancy

for the droplet which means the force exerted on the draining film due to the weight of the droplet will be less, but it will also lead to more rapid film drainage, thus the two effects oppose each other. However, at larger density differences the droplet and/or the interface may become deformed extending the draining film area and thus leading to longer rest-times. When deformation occurs it tends to be predominant over the buoyancy effect. A high interfacial tension would resist deformation, thus reducing rest-times. The data obtained show no simple correlation with $\Delta\rho$ or γ_{ow}.

Table 5.2.3-1

Single droplet stability: the effect of the oil phase density and relative viscosity

Oil Phase	Density Difference between oil & aqueous Phase	Relative Viscosity difference between oil & Aqueous Phase
Perfluorotributylamine	0.89	5.003
Perfluorodecalin	0.93	5.198
Perfluoromethyldecalin	0.97	5.703
Perfluoromethylcyclo-hexane	0.81	0.841
Perfluoro-1,3-dimethyl cyclohexane	0.85	1.019
Perfluorohexane	0.69	-0.206

Note that the minus sign in the above table indicates that perfluorohexane is less viscous than water.

5.2.4 Theoretical models of Droplet Coalescence

Many authors have attempted to correlate the rate of film thinning and droplet deformation to physical parameters such as droplet size, interfacial tension and density difference between the phases. Mathematical analysis has been given for four theoretical models (illustrated in figure32).

It is assumed in type I & II models that the deformation is small and that the effects due to the electrical double layer repulsion, electroviscosity, or an adsorbed film are absent. Then, the time required (t_h) for a film to drain evenly to a given thickness is related to the density difference and interfacial tension by the following expressions, (Charles & Mason, 1960):

$$t_h \alpha \Delta\rho^{-1} \qquad \text{----- type I model; Fig. 32}$$

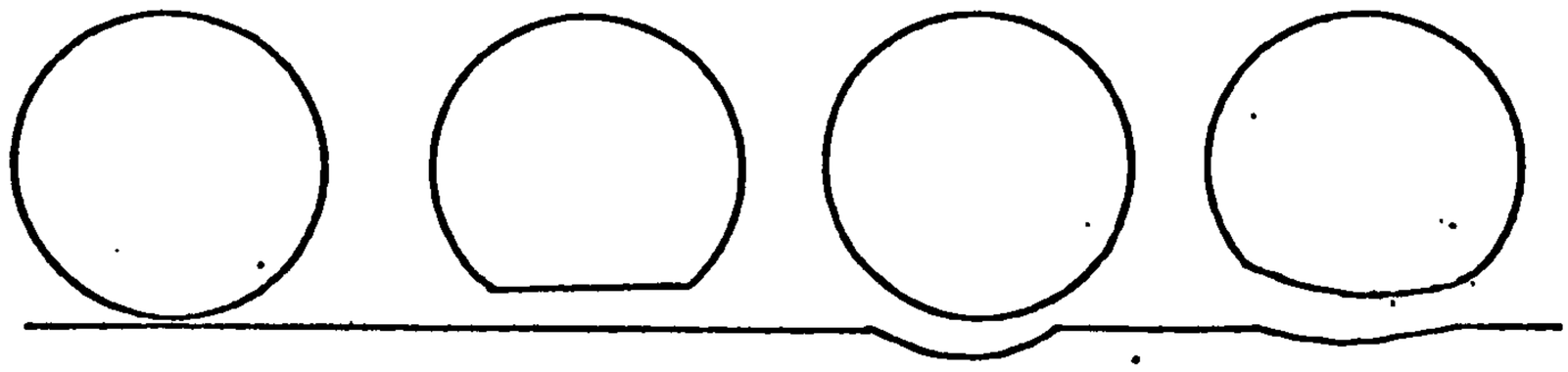
and

$$t_h \alpha \Delta\rho/\gamma_{ow}^{2} \qquad \text{----- type II model; Fig. 32}$$

The rates of film thinning for the models of type III and IV have been interpreted on similar basis to the type II model by a number of authors (Gillespie & Rideal, 1956; Princen, 1963; Chapelear, 1961).

In many instances the theoretical models have been found to be unrepresentative of the real situation. It is thought that the film between a flattened drop and the interface may thin more rapidly around its perimeter causing a lens or "dimple" to form, in the centre. The effect of droplet "dimpling" on film drainage has been reported by Frankel & Mysels, (1962) and Hartland (1967). It is difficult to treat this geometry mathematically to calculate the rate of film thinning. However, calculations of Frankel &

Figure 32 Proposed Models for the Draining Film between a small Droplet and a Liquid-Liquid Interface



Type I : Droplet non-deformable, Interface non-deformable

SPHERICAL-PLANAR MODEL

Type II : Droplet deformable, Interface non-deformable*

PARALLEL PLATE MODEL

Type III: Droplet non-deformable, Interface deformable*

Type IV : Droplet and Interface both deformable*

* Uniform Film Models.

Mysels (1962) show that the drainage rate in the thinnest part of the film is approximately the same as that obtained using the parallel plate model type II. Hartland (1967) found that coalescence occurred more frequently at the thinnest region around the edge of a dimpled film, unless tilting of the droplet caused irregular and rapid thinning of a particular region of the film.

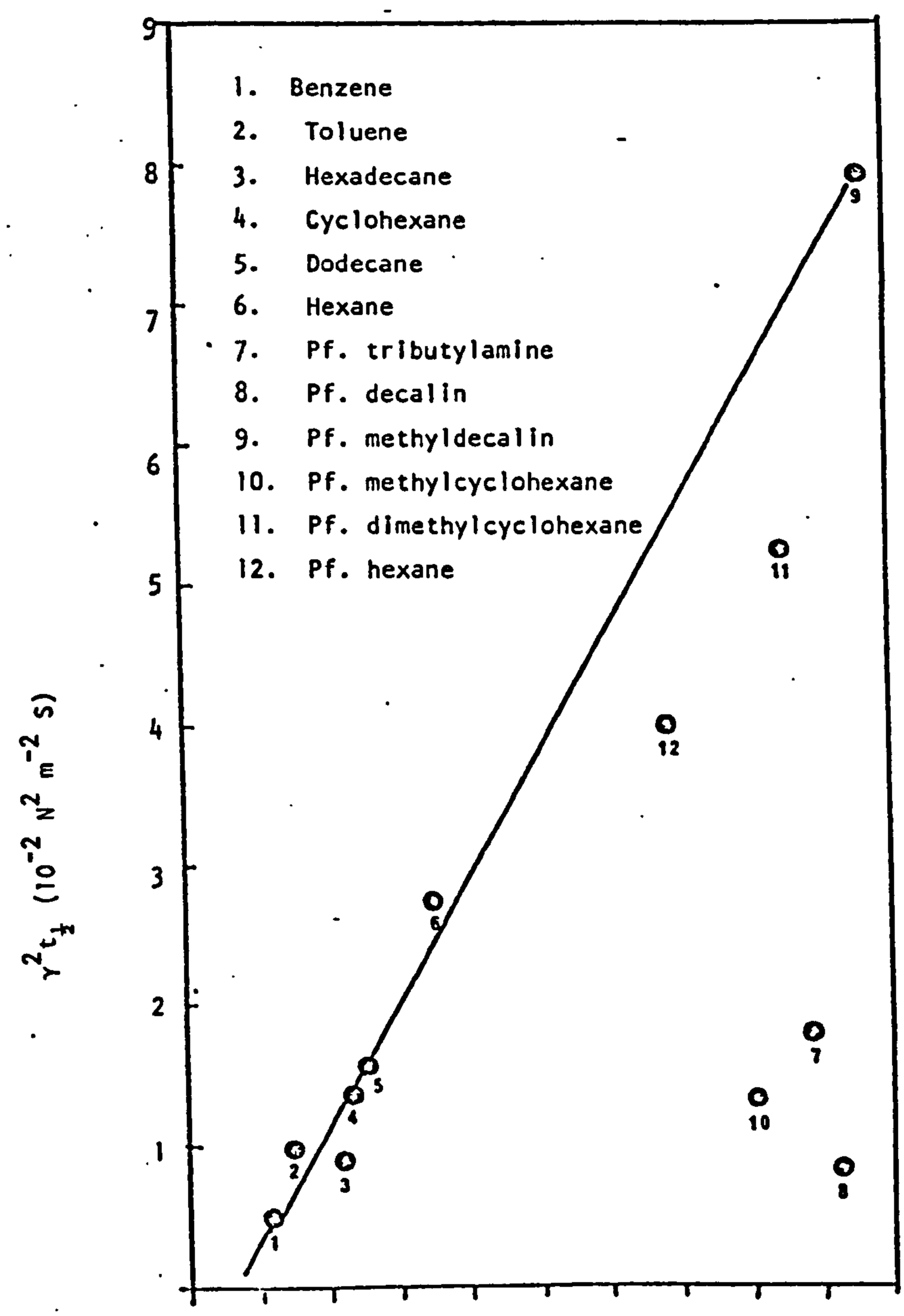
Smith (1975) has found that the parallel plate model gave good correlation of droplet stability of various hydrocarbon oils stabilised by SDDS and that the critical rupture thickness for the alkanes was about 150 nm. A similar correlation has been attempted here (figure33). It is clear that the data for the perfluorochemical oils do not follow the same pattern as the hydrocarbon oils.

5.2.5 The Effect of Additives in the Oil Phase

Addition of small concentrations of another fluorochemical to a fluorocarbon oil phase (at a concentration of 0.05M) exerted no influence on the single droplet rest-times of that oil phase. Similarly no effect of additive was detectable on the relative viscosity or surface or interfacial tensions of the oil phase. Consequently it can be assumed that these additives did not affect the rate of film drainage in the systems concerned.

However, the addition of perfluorohexane to n-hexane had an effect on the droplet stabilities of n-hexane. At lower concentrations it decreased the rest-times but at higher concentrations (0.4M and 0.6M) it prolonged the rest-times of hexane droplets. These two

Figure 33 . Analysis of droplet coalescence data by the uniform film model for film drainage. The hydrocarbon data is taken from Davis et al (1976)



opposing effects from the same chemical are difficult to explain. May be the answer lies in the thermodynamics of binary mixtures. The solubility parameter (δ) gives an estimate of the cohesive nature of the oil phase. But the estimation of adhesive forces between oil molecules is more difficult. Davis & Smith (1973) have proposed that values for the activity coefficient at infinite dilution of the corresponding binary mixtures (e.g. dodecane and perfluorochemical oil) provide a qualitative estimate of adhesive forces. Scott (1958) has reported on the mixtures of perfluorohexane - hexane and perfluoroheptane - heptane. Such mixtures have activity coefficient, at infinite dilution, in the region of 2, an ideal system would have a value of about 1. Thus mixtures of perfluorohexane - hexane were not ideal.

However, the total effect may not only be due to the non-ideality of these mixtures, the density difference between the oil and the aqueous phase may also be important since it affects the drainage of the interfacial film. Addition of perfluorohexane to hexane increases the density of hexane and therefore reduces the density difference between the aqueous phase and the oil phase (see table 4.2.3-2). The hexane droplets were rising owing to the lower density of hexane than water, and resting at the o/w interface before coalescing. The rest-time is affected by the time taken for the interfacial film to drain which, in turn, is affected by the force exerted by the droplet on the film. This force exerted by the droplet is altered by the change in the density difference. Exactly how this alters the droplet rest-time is complex. At lower density differences the film draining area will be less, since dimpling of the droplet and/or the interface would be

unlikely, and therefore the rest-time will be reduced. At high density differences if dimpling occurs the rest-time is likely to be increased because there will be a greater interfacial film area which will have to drain before coalescence can occur. But if there is no dimpling the rest-time of droplets is likely to be reduced because of greater force exerted by the droplet on the interfacial film will be the predominant factor making it more likely to rupture.

5.3 Bulk Emulsion Stability

Bulk emulsion stability determined by particle size analysis and viscosity, shows that the stability of perfluorochemical emulsions depends on both the oil phase and the emulsifier. In the case of SDDS there is good correlation between emulsion stability data and single droplet coalescence rest-times, the order of stability was found to be perfluorodecalin < perfluoromethylcyclohexane ≪ perfluorotributylamine. Since perfluorochemical oils have very low solubility in water the instability due to molecular diffusion (appendix 4) is probably negligible. Therefore we may conclude that coalescence is the major route for emulsion breakdown in these systems.

5.3.1 The Coalescence Process

The coalescence process has already been discussed in detail in section 1 and appendix 1. It has been pointed out that the presence of a suitable surfactant can allow emulsion droplets to lie in close proximity without coalescing i.e. permit flocculation in the secondary minimum but not coagulation in the primary minimum (DLVO theory appendix 1). This kind of stability is provided

typically by polymers and macromolecular stabilisers which form a thick interfacial film thus preventing droplets to approach each other close enough to pass the energy maximum. All the fluorocarbon emulsions showed sedimentation owing to the greater density of the oil phase. The sediment in most cases was easily redispersed by shaking. Hence we can conclude that these systems were flocculated.

In a well stabilised emulsion only a small number of droplet collisions result in coalescence. Under these conditions it has been argued that the rate determining process in the coarsening of emulsions is the film rupture between the adjacent droplets (Van den Tempel, 1958). Schulman & Cockbain (1940) have stated that the most effective stabilising conditions for oil-in-water emulsions are:

(1) that the interfacial film is stable and as closely packed as possible

(2) that the interfacial film is electrically charged.

Becher (1962) has suggested that the coalescence mechanism involves rearrangement of surfactant molecules during droplet contact, allowing closer approach and coalescence. Cockbain & McRoberts (1953) also suggested that displacement of the interfacial film was necessary for coalescence. They thought that wetting of the adsorbed species by dispersed phase determined the probability of droplet coalescence. Sumner (1957) envisaged a similar process although the oil was thought to penetrate through the adsorbed film between adjacent droplets. MacRitchie (1967) has related the tendency of surfactant desorption into oil or continuous phase with resistance

to coalescence. Suzuki & Higuchi (1970) have suggested that in the case of charged surfactant molecules, lateral displacement can occur due to interionic repulsion, although this would be opposed by interfacial tension gradients.

Van den Tempel (1958) has shown that droplets in contact are still separated by a water of solvation film at least 10 nm thick. Since then, Florence & Rogers (1971) have postulated that the major effect of emulsion stabilisers is to decrease the probability of rupture of the aqueous film. Recently, more emphasis has been placed on the properties and stability of thin films in relation to emulsion stability (Ottewill & Buscall, 1976). However, there is still a need for in depth study of the mechanism of thinning of aqueous films in oil.

5.3.2 The Significance of Sedimentation

Sedimentation of emulsion droplets is analogous to creaming. All the fluorocarbon emulsions exhibited sedimentation. In the sediment the percentage by volume of dispersed phase is greater. The heterogeneity of particle size of an emulsion can permit close packing of droplets. Flattening of droplets against each other will reduce the sediment volume whilst the presence of lamellae between adjacent droplets will tend to increase the sediment volume. The droplets, under normal conditions, will be loosely packed and the sediment will have a further quantity of the continuous phase trapped in it. The exact contribution of these effects towards emulsion stability is not fully understood. The probability of coalescence of droplets in the sediment is greater than in the same non-sedimented system. The sediment of all the fluorocarbon

emulsion systems could easily be redispersed by shaking the container which implies that the emulsion droplets were flocculated in the secondary minimum (DLVO theory, appendix 1).

5.3.3 The Effect of the Oil Phase

The emulsion stability and the initial droplet diameter was dependent on the oil phase for a given emulsifier. Perfluorodecalin gave the smallest droplet size but very poor stability where as perfluorotributylamine gave most stable emulsions but slightly larger initial particle size. Thus, contrary to previous literature reports (Geyer, 1975) it can be stated that perfluorodecalin is easy to emulsify and produces emulsions of fine particle size but these particles are very unstable except when a polymeric emulsifier such as Pluronic F-68 is used.

The differences in stability may be explained by differences in surfactant adsorption caused by the differences in intermolecular forces. Let us consider the adsorption of SDDS to the o/w interface. It will be the resultant effect of two processes (Hutchinson, 1948); separation of oil molecules as the molecules of surface active agents penetrate the oil phase (cohesive forces); interaction between hydrocarbon chains of the surfactant and molecules of the oil phase (adhesive forces). When the adhesive forces are greater than the cohesive forces the surfactant "tail-ends" will readily penetrate the oil phase. The cohesive nature of the oil phase can be estimated from its solubility parameter (which is related to the heat of vaporisation and solvent molar volume), (Davis & Smith, 1973). Perfluorocarbon oils have low solubility parameters, (in the region of 6 to 7), compared with hydrocarbons which have

higher values (about 7 to 9) (Hildebrand & Scott, 1962). Thus the cohesive forces between fluorocarbon molecules are low which is reflected in their low surface tensions (about $20mNm^{-1}$ or less). The estimation of adhesive forces between the molecules of surfactant and oil is more difficult. Davis & Smith (1973) have proposed that values for the activity coefficient at infinite dilution ($f\infty$) of the corresponding binary hydrocarbon mixtures (e.g. dodecane and a perfluorochemical oil) provide a qualitative estimate. Such values are limited in the literature but Scott and his colleagues (1958) have studied a number of mixtures of aliphatic hydrocarbons. Such mixtures have positive excess free energies. For perfluorohexane - hexane and perfluoroheptane - heptane mixtures $f\infty$ is in the region of 2.0. An ideal system (e.g. dodecane in hexadecane) would have $f\infty = 1.0$.

Thus, for perfluorochemical oils both cohesive and adhesive forces are very low. The poor adhesion between surfactant chain and oil will result in poor penetration of SDDS and hence the emulsion will have poor stability. The extent of penetration will be oil dependent and since different oils will have different polarities, there will be differences in the emulsion stability. This is also true when a nonionic polymeric material such as Pluronic F-68 is used, even though, in this case, the effective penetration of the oil phase is not a prerequisite of adsorption to the interface. This view is confirmed by the bulk emulsion stability results which show that once again the stability is not independent of the nature of the oil for once again we see that stability increases as we pass from perfluorodecalin to perfluorotributylamine. Secondary variables such as density difference and interfacial tension also play a part.

5.3.3.1 The Effect of Additives in the Oil Phase

The bulk emulsion stability of a given oil was increased if a small amount of an additive, which itself formed more stable emulsions than the oil under test, was incorporated into the oil phase. This effect was illustrated by the preparation of n-hexane emulsions where a small amount of a fluorocarbon oil was incorporated into the n-hexane oil phase. Perfluorotributylamine had the most stabilising effect out of the fluorocarbon additives investigated.

It is postulated that in these emulsion systems the mechanism for emulsion breaking is predominantly molecular diffusion. The addition of a small amount of a fluorocarbon oil to n-hexane retards molecular diffusion because the aqueous solubility of fluorocarbons is markedly less than the water solubility of hexane. However, exactly how a small amount of an additive can achieve this effect is not fully understood. One theory is that the additive concentrates at the interface but interfacial tension measurements contradict this view since there is no change in the interfacial tension in the presence of the additive at the same concentration as that utilised in the emulsion systems. The effect of the additive may be a resultant of a number of effects, for example it could alter the wetting properties of the adsorbed species by the dispersed phase, it could affect the solvation envelope around the emulsion droplets which acts as a coalescence barrier, or it could affect the viscosity of the interfacial film and hence its drainage. But the question still remains exactly how are these effects brought about? Maybe the diffusion of hexane across the oil-water interface is altered by the additive. The answer must lie

in the intermolecular forces involved. A more detailed study in this direction should be useful.

5.3.4 The Effect of Surfactant

The results show that the emulsions stabilised by a surfactant system (that is the use of two or more surfactants, e.g. Pluronic F-68 and lecithin, in combination) coarsened at slower rate on storage than the emulsions containing a single emulsifier. However, the emulsifier system had to be chosen carefully so that the surfactants were compatible with each other, for example it was found that emulsions prepared by using a combination of F.C.170 with Pluronic F-68 were less stable than if either of the surfactants were utilised alone. It was found that on mixing these two surfactants an insoluble precipitate was formed, thus less surfactant was available for stabilising the emulsion droplets against coalescence. Best results, from the stability point of view, were obtained when a small molecular weight surfactant was used coupled with a suitable large molecular weight, polymeric type, emulsifier, e.g. lecithin and Pluronic F-68. This is in agreement with the work reported by Tadros (1974) using hydrocarbon emulsion systems. He postulated that the two surfactants formed a complex which was a better stabiliser than either agent alone. Probably the low molecular weight material will lower the interfacial tension so as to aid emulsification and will diffuse rapidly to the interface, forming a relatively stable interface and thus preventing coalescence at short times. This will allow the larger molecular weight emulsifier enough time to diffuse to the interface and give longer term stability on storage. This argument is supported by the fact that when a small molecular weight

surfactant was used alone emulsions of small initial particle size were obtained which were relatively unstable on prolonged storage whereas emulsions prepared from large molecular weight emulsifiers had relatively large initial droplet diameter but were more stable on prolonged storage. Therefore a combination of the two types should give the best of both worlds. An additional advantage of using an emulsifier system was that an emulsion of relatively narrow particle size distribution was obtained.

Pluronic F-68 gives good stability as would be expected from a polymeric material because polymers can give steric stabilisation against coalescence (see DLVO theory appendix 1). The mixed fluorinated surfactant combination was disappointing. It was expected that this system would give good stability since the forces of adhesion between the fluorocarbon tail of the surfactant and the various fluorocarbon oils should have been significant. However, the order of stability was as for SDDS (Perfluorodecalin ≤ perfluoromethylcyclohexane < perfluorotributylamine).

Emulsions stabilised by egg lecithin were also relatively unstable. The relative size changes are similar to those for the SDDS systems but the positions of perfluorotributylamine and perfluoromethyl-cyclohexane are reversed at 57 days. Lecithin is a naturally occurring emulsifying agent, similar in structure to a fat, however, instead of being a simple triglyceride, one position on the triglyceride function is occupied by a substituted phosphoric acid (Becher, 1965). Egg lecithin is used as the emulsifier in intravenous fat emulsions for parenteral nutrition. The oils in this case are of vegetable origin (soya, cotton seed) and emulsions of small particle size and long term

storage stability can be formulated (Davis, 1974). The poor stability of lecithin stabilised perfluorochemical emulsions may be attributed to the poor affinity between the hydrophobic regions of the emulsifier (long chain fatty acid residues) and the oil.

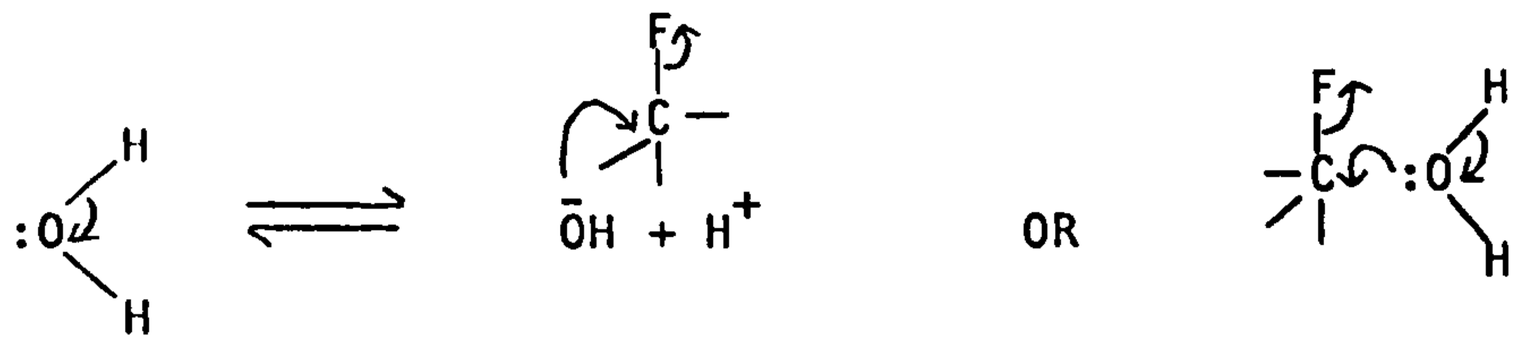
The droplet sizes immediately after emulsification show a rather different picture. SDDS produced larger droplets whereas lecithin and some fluorochemical surfactants produced some of the smallest. Pluronic F-68 was in between.

5.4 The Fluoride-ion Problem

The results clearly show that fluoride ions are produced during the preparation of fluorocarbon emulsions by sonication. When the sonication time is long the fluoride concentration may become high enough to cause death on infusion of the emulsion into a living system. It may also adversely affect emulsion stability on storage. Clark and co-workers have reported (1975) that fluoride ions can act synergistically with anaesthetics and other factors which may result in a markedly lowered cardiac output. The production of fluoride ions means that other degradation products may also be produced, which may have their own adverse effects and, unlike the fluoride ions, cannot be removed by ion exchange resins.

The results also show that the presence of carbon dioxide gas atmosphere during sonication markedly reduces the F^- ion production. Other inert gases such as nitrogen have no effect. It is postulated that carbon dioxide may inhibit the fluoride ion production by the following mechanism:

The ultrasonic energy input weakens the C-F bond so that, in the absence of any carbon dioxide, nucleophilic attack by ^{-}OH could occur:



Now, in the presence of carbon dioxide, the CO_2 dissolves in the aqueous phase thus acidifying it. Therefore there would be less likelihood of nucleophiles being present and there is more likelihood of electrophiles such as $H-\overset{+}{\underset{H}{O}}-H$

The above argument is supported by the fact that other gases were found to have no effect and that sonication in the absence of any water did not produce any fluoride ions. Furthermore, perfluorotributylamine gave rise to more fluoride ions than perfluorodecalin (see table 4.3.6-1) for a given sonication time. This is not contradictory to the above argument, indeed one would expect this result because of the greater number of fluorine atoms in the perfluorotributylamine molecule, these being more electronegative withdraw electrons by inductive effect thus making the molecule more likely to undergo nucleophilic attack.

5.5 Sterilisation and Storage of Fluorocarbon Emulsions

The methods for sterilisation may be divided into two groups, namely, Physical and Chemical. Physical methods include heat (moist or dry), ultra-violet light, ionizing radiation and

filtration through a bacteria-proof filter. Chemical methods include the use of liquid or gaseous sterilising agents. As far as fluorocarbon emulsions are concerned, chemical methods cannot be used because of toxicity problems. From the physical methods the use of ultra-violet light can be excluded owing to its poor penetrability. Ionizing radiation was not chosen because of poor penetration of radiation (e.g. from β emitters) and requirement of special equipment. The use of dry heat involves relatively high temperatures which could be detrimental to the stability of the product. Therefore moist heat sterilisation (Autoclaving) and sterilisation by filtration were investigated.

5.5.1 Autoclaving

Moist heat is thought to destroy micro-organisms by causing protein denaturation (Rahn, 1945), and it has been shown that moist heat kills micro-organisms at lower temperatures and in shorter times than dry heat. For example, very few vegetative bacteria can survive 10 minutes at 80°C, but spores of pathogens require 30 minutes at 115°C; most viruses have similar susceptibility to heat as vegetative bacteria.

The results show that autoclaving at a temperature of 126°C for 15 minutes produced sterile fluorocarbon emulsions. The particle size data show that sterilised emulsions coarsened at a similar rate to non-sterile emulsions when stored at room temperature. However, immediately after sterilisation the mean particle diameter of the sterilised emulsions was greater than that of the non-sterile ones. Therefore it may be concluded that exposure to elevated temperature during autoclaving accelerates

coalescence.

5.5.2 Filtration

Sterilisation by filtration requires a filter in which the pores are smaller than the bacteria. This would ensure 100 per cent efficiency. For the sterilisation of fluorocarbon emulsions a milipore filter having 0.45 μ pore size was found to be satisfactory (milipore filters of smaller pore size are available). Straining is not the only way in which small particles may be removed by a filter, the majority can be retained by intermolecular forces between the pore surfaces and the particles.

Although sterile fluorocarbon emulsions were obtained by this method, a number of disadvantages were encountered. The filter choked very quickly and GLC analysis showed that up to 20% of the original weight of fluorocarbon was lost during filtration. This would suggest that larger particles had been filtered out; this was confirmed by particle size data of the filtered emulsion. These disadvantages would have been even greater if a smaller pore size filter was used. An additional disadvantage is that special precautions have to be taken during the procedure such as the need of aseptic conditions (sterile air cabinet was needed during the procedure). This makes the method cumbersome. For these reasons autoclaving was the preferred method.

5.5.3 Sterilisation by Autoclaving Coupled with Filtration

It is generally thought that if a liquid preparation is to be sterilised by autoclaving there is no need for prior filtration to remove micro-organisms or their spores. However, since the

stability of an emulsion is adversely affected by exposure to high temperature, any pretreatment of the preparation, which will result in a lowered temperature and exposure time necessary for sterility, will be advantageous provided it does not increase the toxicity; (the use of a bactericide may increase toxicity of a fluorocarbon artificial blood preparation; however, inclusion of an antibiotic may be advantageous provided it does not reduce the stability of the preparation). Another factor to consider is that when a population of living micro-organisms is heated at a lethal temperature the cells are not all killed at the same time, the number of survivors falls gradually with exposure time. If a certain fraction of initial number is destroyed in a given time interval then the same fraction of survivors will be destroyed in the same succeeding time interval and a plot of number of survivors versus time shows an exponential death rate. However, deviations from exponential death rate have been reported (Jordan, Jacobs & Davis, 1947). Therefore it would be logical to assume that if the initial number of contaminants is small (e.g. after filtration) the number of survivors after a given exposure time to lethal heat would be smaller and hence the exposure time necessary for sterility would be less.

The results of the research herein confirm the above arguments. Therefore it is concluded that fluorocarbon emulsions should be sterilised by filtration, through a milipore filter (0.45 μ pore size) of aqueous and oil phases, prior to emulsification, followed by autoclaving for a shorter time than usual. The lowered sterilisation temperature and shorter exposure time also means that any decomposition of the surfactant will be minimal. The solubility in water of Pluronic F-68 decreases with increasing temperature, therefore the emulsions stabilised with it may be more likely to

undergo degradation at higher temperatures and therefore a lower sterilisation temperature and shorter exposure time would be advantageous. Alternatively the emulsifier may form a solid film around the emulsion droplets and the stability may not be adversely affected.

5.5.4 Sterility Tests

In the United Kingdom sterility tests are controlled by the British Pharmacopoeia and the Therapeutic Substances Regulations, but additional advice is given in a report of a study group on "General Requirements for the Sterility of Biological Substances" published by the World Health Organisation (1960) (commonly known as the WHO report).

Sterility, in the microbiological sense, means freedom from living micro-organisms and therefore, strictly speaking, to claim that a batch of product is sterile means testing the entire contents of every container in the batch using a test which provides optimum conditions for the growth and multiplication of every organism, vegetative or spore, healthy or injured, that might be a contaminant. Unfortunately neither of these conditions can be satisfied. Consequently, sterility tests can only show that organisms capable of growing in the test media under the selected conditions are absent from the fraction of the product that has been tested. Therefore sterility testing should not be used as the sole means of controlling sterile processing. Heat sterilisation methods can be checked bacteriologically and instrumentally and procedures involving asepsis can be checked by careful supervision of the sterile air cabinet. To obtain reliable data from sterility tests it is essential to take sufficient samples, use sensitive culture media and during testing reduce accidental contamination to a minimum.

The sterility tests used complied with the Therapeutic Substances Regulations that is the sample size was at least 0.1% of the total volume of emulsion under test or a minimum of 1 ml. Two types of Culture media were utilised. Nutrient broth for aerobic bacteria and thioglycollate medium for anaerobes. The thioglycollate medium consisted of:

Reducing agents - sodium thioglycollate and dextrose;

resazurin as an oxidation reduction indicator;

Nutrients, consisting of:

Pancreatic digest of Caesein (nitrogen source)

dextrose (carbon source)

yeast extract (growth factor source)

Sodium Chloride.

This medium is suitable for detecting anaerobes provided not more than 30% of the upper region is coloured.

The incubation conditions were in compliance with the B.P. and U.S.P. specifications. Additional samples were incubated at $4^{o}C$, room temperature and at $50^{o}C$ to simulate storage conditions of emulsions at these temperatures. Controls were included for each test which included the same volume of emulsion as the tests.

All the sterility tests were negative except the controls which showed growth indicating that the methods used for sterilising the fluorocarbon emulsions were successful. The volume of nutrient media used was 50 ml in each case to ensure that any growth inhibitory effect of the emulsion sample was diluted out. Furthermore the Controls contained the same volume of emulsion sample as the tests to prove that the growth media were capable

of supporting microbial growth in the presence of the emulsion sample.

5.5.5 Storage

When considering storage of fluorocarbon emulsions intended to be injected intravenously, not only the storage temperature but also the type of container used are of importance. The container material should not affect the contents. Some types of glass yield appreciable amounts of alkali or shed flakes while plasticisers, catalysts and other additives, used during manufacture of plastics, may leach out of certain plastic containers. The container should be strong enough to withstand the temperature and pressure changes associated with sterilisation by autoclaving. It should be easy to clean or cheap enough to discard when empty. To allow visual examination of the contents for particles and signs of decomposition it should be transparent and colourless, and it should maintain its transparency for several years' storage under a variety of climatic conditions. Although specialised applications have been found for some plastics, glass is still the favourite material for such containers.

The effect of temperature on the stability was also investigated and the results show that the storage temperature for optimum stability of these emulsions is about $4^{o}C$, i.e. storage in a refrigerator. But it is important not to allow the emulsions to freeze since freezing and subsequent thawing destroys the emulsion. The lowered temperature may result in a decreased solubility of the surfactant which may adversely affect the emulsion stability, but on the other hand a lowered temperature

means the system possesses less energy and collisions between particles due to Brownian motion will be fewer and therefore rate of coalescence will be retarded. An additional effect will be that the viscosity of the draining film is likely to be higher at lower temperature which will also enhance stability. Which effect is predominant will depend on the given emulsion system. Therefore, each emulsion system, theoretically, will have a different optimum storage temperature. In the case of emulsions stabilised by Pluronic F-68 the problem of decreased surfactant solubility with lowered temperature does not exist since its solubility increases with decreasing temperature.

The results show that the emulsions stored at 50°C coarsened more rapidly than those at lower temperatures. This is not surprising since increasing the temperature causes changes in most of the factors which could affect stability. The bulk viscosity, interfacial tension, and adsorption at the interface all decrease while the potential energy (kT) and the Brownian velocity both increase. Double layer potentials can either increase or decrease, according to the sign of the surface potential. The Gouy theory gives the expression:

$$\frac{d\Psi_o}{dT} = \frac{\Psi_o}{2T} - \frac{R}{e} \tanh \left(\frac{e\Psi_o}{2KT}\right)$$

in which the first term on the right hand side of the equation is larger than the second term. Dispersion interactions are usually regarded as independent of temperature, although recent work indicates that this may not be true for certain systems (Parsegian & Ninham, 1970) in which infrared contributions are appreciable. The overall effect of increased temperature on emulsions usually results in decreased stability.

Shinoda and Saito (1969) have studied the temperature dependence of emulsion stability; they took as measures of stability the mean droplet size as a function of time. They established the fact that stability decreased very rapidly as the phase inversion temperature (PIT) was approached. They also suggested that the stability was related to the difference between the system temperature and the PIT. This idea has been supported by Parkinson & Sherman (1972).

The theory behind PIT is as follows: any mechanism for phase inversion must involve a coalescence process at one stage, (Clowes, 1916; Schulman & Cockbain, 1940), so that inversion is analogous to coalescence in that respect. In an unstirred emulsion inversion can be envisaged as the rapid coalescence of a cluster of coagulated droplets, which leads in the case of o/w emulsions, to the formation of a water droplet in an oil continuous phase, the process must be rapid and at the PIT this is so. Therefore, in principle, the PIT should be predictable, but this has not yet been done. In sheared systems the situation is different since both o/w and w/o emulsions are formed simultaneously and type of emulsion which results at the end of the process depends on the relative rates of coalescence of the two types of the newly formed emulsions. Davies has discussed this situation in detail (Davies, 1957 and 1960). Coalescence is still important but other factors such as orthokinetic coagulation effects will also affect the process. Therefore sheared systems would be expected to have different PIT compared with unsheared systems.

The PIT of a system can easily be determined experimentally, unless

it is close or beyond a phase transition of one of the emulsion components, and may be used to assess stability. Correlations have been made between PIT method for measuring stability and the more traditional method of hydrophile - lipophile balance (HLB), (Vold, 1972, Bierre, 1971). Parkinson et al (1972) found that for series of emulsions stabilised by surfactants of varying HLB, systems having maximum PIT also had an HLB corresponding to maximum stability. Shinoda & Saito (1969) have claimed that the PIT method should give a more sensitive measure of stability of emulsions, although this is not apparent from the results of Parkinson et al. However, exceptions to the PIT rule have not yet been reported. But Enever (1976) has sounded a note of caution regarding the use of PIT to predict long-term stability. He concluded "Although phase inversion temperature is a valuable method for ranking the emulsions in order of relative stability, its general applicability for predicting long-term stability is limited when phase changes occur on heating"; such as the disappearance of the viscous liquid crystalline phase on heating which at lower temperatures would have a considerable stabilising influence.

5.6 Phagocytosis of Fluorocarbon Emulsions

The results show that the in-vitro rate of phagocytosis of fluorocarbon emulsion particles is dependent on the particle size of emulsion droplets and the nature of the surface of the emulsion droplets. Emulsions with a smaller mean particle diameter were ingested by phagocytes at a slower rate compared with the one having a larger mean particle diameter, (the mean particle diameter in all cases was submicron). The emulsions prepared with non-ionic emulsifiers were taken up by the cells more rapidly than those with

ionic emulsifiers.

These results are in agreement with the studies of Davies & Thomas (1975) and Stossel et al (1972). These workers used latex particles to study the factors affecting the in-vitro phagocytosis of particles. The results also correlate, qualitatively, with the clearance of fluorocarbon emulsions from the circulation of animals. For example, Yokoyama et al (1975) have reported that the fluorocarbon emulsions having a smaller mean particle diameter (less than 0.2 μm) were cleared from the circulation of rats at a slower rate than the emulsions having a relatively larger mean particle diameter (about 0.5 μm).

Davies & Thomas (1975) have postulated that in the presence of serum there are two different mechanisms of particle-cell contact. They found that when the serum concentration was insufficient to produce a gamma globulin monolayer around the particle, most of the uptake by phagocytes took place at zero time and was accompanied by extensive clumping. They concluded that "this type of uptake cannot be regarded as phagocytic ingestion and must, therefore, be interpreted as spontaneous adherence of cells to particles". They also found that after the formation of the gamma globulin monolayer no uptake took place at zero time and uptake increased gradually with time. Microscopic examination revealed very little clumping and the cells appeared to have ingested particles. Therefore they concluded that this represented phagocytosis pursuant to recognition by the cell.

Our phagocytosis experiments did not distinguish between the decrease

in number of particles of emulsion due to adherence of particles to the phagocytes and ingestion of particles by the cells. But this may be insignificant because the process of phagocytosis probably consists of two main processes, one the adherence of particle to the cell (this would be necesary for the cell to recognise the particle as foreign) and second the subsequent ingestion of the particle by the cell. Any decrease in the number of particles due to coalescence of the emulsion droplets was taken into account by having a control which differed from the samples only in as much that it did not contain any phagocytes. However, it should be remembered that the in-vitro phagocytosis of fluorocarbon emulsions was carried out in the absence of serum and therefore the mechanism of phagocytosis is likely to be different from that operating in-vivo, consequently the rate of clearance of emulsion droplets will be different in-vitro compared with in-vivo. Despite the lack of quantitative correlation with the in-vivo data, the in-vitro phagocytosis is useful to investigate qualitatively the factors which affect the rate of phagocytosis.

Geyer (1967) has investigated the clearance of fat emulsion particles from the blood stream, and the effect of orally administered fat on the removal of intravenously injected fat from blood. The emulsions used were of the Lipomul IV type (Upjohn) containing cottonseed or coconut oil, or cottonseed oil emulsions (10% oil phase) stabilised by various Pluronics. He found that with the increase in the molecular weight of the Pluronic the rate of removal of emulsion from the blood decreased. Singer et al (1967) have studied the in-vivo phagocytosis using Radioiodinated latex particles and have concluded that "Particles in-vivo will

interact with plasma constituents according to the properties acquired from the coating or stabilising agent, and relative stability will be similar to that observed in vitro". Wilkins (1967) has studied the recognition of foreign from native particles, by the phagocytes, from the surface chemistry point of view. He has concluded that the recognition and the subsequent ingestion of foreign particles by phagocytes are two distinct processes. The nature of the particle surface will affect the recognition process and therefore the phagocytosis. If we could control the surface characteristics of particles it may be possible to formulate preparations with predictable rates of clearance from the blood stream. Furthermore, it may be possible to disguise emulsion droplets such that the phagocytes treat them as native particles. This would be very useful in the case of fluorocarbon artificial blood substitutes. Further studies to find out the effect of colloidal factors on the processes of recognition and ingestion by phagocytes would provide useful data.

5.7 Oxygen Solubility and Release

The fact that oxygen is highly soluble in liquid perfluorochemicals has been well documented. Clark in 1966 demonstrated it by immersing live mice into aerated liquid perfluorochemicals, which survived. Normal saline and blood plasma dissolve about 3 per cent oxygen by volume, whole blood about 20 per cent, compared with 40 per cent or more for pure perfluorochemicals. Carbon-dioxide solubility is about twice as much as oxygen in perfluorochemicals.

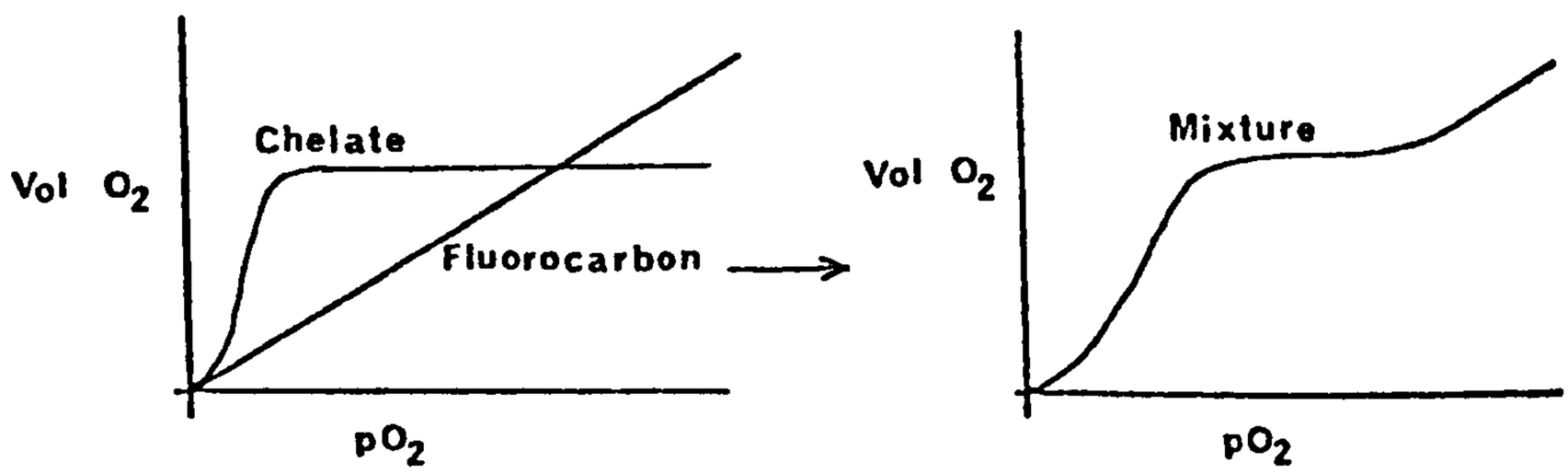
Emulsions of perfluorochemicals can also function as oxygen transporting media. Miura et al (1973) have studied the kinetics

of oxygen uptake and release by the oil-in-water emulsions of perfluorotributylamine with either Pluronic F-68 or phospholipid as the emulsifier. They found that the oxygen uptake and release of the emulsion follows first order kinetics and is reversible. Oxygen uptake by the perfluorotributylamine droplets was found to be very rapid, attaining equilibrium within ½ second, however, our results show that equilibrium value is reached within 1 second with about 75% of the dissolved oxygen being released within ½ second; even so, the release rate is quite rapid.

It has been demonstrated that fluorocarbon emulsions can be used to completely replace the blood of animals (Geyer, 1968 and 1975) or to perfuse isolated organs such as the brain, kidney, liver, heart (Sloviter, 1967; Clark et al, 1975). One point to remember is that the oxygen solubility in fluorocarbons is directly proportional to the partial presssure of oxygen and past experiments in which the blood of animals was completely replaced with an emulsion, involved the use of 100% oxygen atmosphere (hyperbaric chamber) to keep the animals alive. The major disadvantage of oxygen rich or pure oxygen atmosphere is the risk of fire. However, recently, it has been reported that provided a non-toxic chelating agent could be synthesised to act as an oxygen carrier in a similar manner to haemoglobin, it could be added to a fluorocarbon emulsion thus combining the linear oxygen absorption behaviour of a fluorocarbon with the experimental behaviour of a chelating agent. This combination would provide an overall behaviour such as to avoid the need for high oxygen tensions as illustrated in figure 34, (Baldwin, 1975). It has been reported that there is now emerging, in the field of chelating agents, the possibility of synthesis of

Figure 34. Oxygen uptake Effect of Chelating agent added to the Fluorocarbon.

Note that pO_2 stands for partial pressure of oxygen.



iron based oxygen carriers with chemical structure based on the porphyrin ring, (Baldwin, 1975). These, if proved to be nontoxic and stable, would enormously enhance the likelihood of an artificial blood being generally used in the future. Geyer (1976) has discussed in detail the design of artificial blood substitutes. He concludes that provided the stability problems can be overcome, it would be possible to include such additives as antibiotics and nutrients in these preparations, as well as fulfilling the oxygen transport function.

5.8 Conclusions

In the previous 15 years much work on fluorocarbon emulsions as artificial blood substitutes has been reported. However, most of these reports have been concerned with toxicology of the emulsions and the colloidal aspects had been neglected. In the present work some of the colloidal aspects affecting emulsion stability have been investigated. Three techniques were employed to assess the effect of the various factors affecting stability. The techniques were: measurement of interfacial tension, the stability of oil droplets at the plane oil-aqueous surfactant solution interface, and the bulk emulsion stability determined by changes in the particle size parameters obtained from particle size analysis by electronmicrography.

Van Voorst Vader (1960) has reported that the molecular structure of an oil-water interface is affected by the chemical nature of the oil phase, particularly in polar oils. The results herein show that even though fluorocarbon oils are relatively nonpolar their chemical nature still affects the oil-water interfacial properties,

enough to influence the surfactant adsorption. The area per molecule of adsorbed surfactant clearly demonstrates this.

Very small concentrations (less than 0.1M) of one fluorocarbon oil in another do not appear to affect the interfacial properties of the solvent oil, but the bulk emulsion stability is affected. In the system of perfluorohexane dissolved in n-hexane, it is apparent that at very small concentrations (less than 0.1M) of perfluorohexane the interfacial tension against SDDS solutions is unaffected, at concentrations of about 0.4M there is an increase in the interfacial tension, whereas solutions of perfluorohexane at a concentration of 0.6M in n-hexane show a decreased interfacial tension. It could be that different types of interaction between the perfluorohexane and n-hexane molecules are involved which are concentration dependent. At higher concentrations the perfluorohexane may act as a surfactant. At lower concentrations it makes the solution behave as if it were perfluorohexane. The exact mechanism involved is not understood. A more detailed study of fluorocarbon oil mixtures is proposed in order to gain better understanding of the mechanisms involved.

It is clear from the bulk emulsion stability data that the stability of perfluorochemical emulsions intended for intravenous administration as red blood cell substitutes is dependent on the nature of the oil and the emulsifier. The polymeric agent, Pluronic F-68 provided emulsions with good storage stability, whereas lecithin gave emulsions of small initial particle size which coarsened on storage. Similarly fluorocarbon surface active agents formed emulsions of small initial droplet size but gave poor stability on prolonged

storage. Out of the oils investigated perfluorotributylamine gave more stable emulsions which was attributed to its more branched structure and the presence of the nitrogen atom in the molecule. The use of perfluorotributylamine and an emulsifier system consisting of Pluronic F-68 and lecithin produced the most stable emulsions. Therefore it is proposed that a combined emulsifier system be used to prepare fluorocarbon emulsions intended for intravenous administration. It has the additional advantage of having a narrower particle size distribution. A more detailed study should give rise to other, possibly better, emulsifier systems suitable for preparing fluorocarbon emulsions.

The differences in stability of single droplets at plane interfaces and of bulk emulsions can be rationalised in terms of the intermolecular forces between oil molecules and between oil and surfactant molecules. There is qualitative correlation between single droplet stability at the plane oil-water interface and bulk emulsion stability, in Perfluorochemical oil systems, but exceptions are encountered. Therefore the droplet stability measurements did not provide a priori indication of emulsion stability, but we can conclude that although a system giving good single droplet stability does not necessarily mean good bulk emulsion stability, a system giving poor single droplet stability does imply that the bulk emulsion stability of that system will also be poor. The coalescence rate of droplets at a plane interface is dependent on many factors, including temperature, vibrations, droplet size and interface curvature, but these factors can be carefully controlled to study the effect of the oil phase and emulsifier. The single droplet stability is dependent on the emulsifier concentration and the nature

of the oil phase.

Forced coalescence of emulsion droplets in a centrifuge did not provide a sensible means of predicting bulk emulsion stability. This is not surprising since different conditions operate during centrifuging as compared with storage at normal gravity conditions. However, the effect of the oil phase was the same as that for bulk stability under normal conditions.

Autoclaving was preferred to filtration for sterilising the emulsions because it involved no loss of material, but autoclaving coupled with filtration of constituents, prior to emulsification, was least detrimental to emulsion stability because lower sterilising temperature could be used. Therefore autoclaving combined with filtration is recommended for sterilising fluorocarbon emulsions.

The in-vitro phagocytosis rate of fluorocarbon emulsion droplets depends on the droplet diameter and the nature of the droplet surface. A more detailed study of factors affecting phagocytosis of fluorocarbon emulsions is suggested since it is the main mechanism of clearance of the emulsion droplets from circulation in the body of animals. The oxygen uptake by fluorocarbon emulsions and subsequent release is extremely rapid, reaching equilibrium within half a second to one second.

The fluoride ions which are produced if the emulsion is prepared by sonication can be minimised by sonicating in an enclosed atmosphere of carbon-dioxide.

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Appendix 1

The DLVO Theory

Carroll (1976) has discussed the DLVO theory and its modified forms. The DLVO theory resulted from the work of Verwey & Overbeek (1948) and Derjaguin & Landau (1941) on colloidal systems.

When water is the continuous phase, it is generally true that because of ionization of material at the interface, or the adsorption of ions, all particles possess an electrical double layer which, on interaction with the double layer of other particles, results in a nett repulsion force. These double layer forces when combined with the Van der Waal's attractive forces give the nett interaction force between particles. Both, attractive and repulsive, interactions are functions of the distance between the interacting particles, and the sign of the nett interaction force often depends on the interparticle separation.

If we quantify these forces, the repulsive (double layer) interactions between two particles whose centres are a distance $r = x + 2a$ apart are given by Derjaguin's (1940) approximate expression:

$$V_R = \frac{2\pi \times 10^{-2}}{9} \varepsilon \varepsilon_o a \psi_o^2 \ln [1 + \exp (kx)] \text{-------(1)}$$

Where V_R = repulsive interaction

$\varepsilon_o \varepsilon$ = the permitivity of vacuum and medium respectively

ψ_o = electric potential at the surface

x = distance between interacting particles

k = Boltzeman constant

a = Particle radius.

Strictly speaking equation 1 should be used for low values of ψ_o and when $Ka \gg 1$.

The forces of attraction between the particles, postulated by Van der Waals, also result from electrical interactions. Three types of such attraction are recognised:

1. Two permanent dipoles mutually orientate each other in a way so that nett attraction results.
2. Dipolar molecules induce dipoles in neighbouring molecules so that attraction results.
3. Attractive forces are also operative between non-polar molecules. These universal attractive forces (known as dispersion forces) were first explained by London (1930) and are caused by polarisaton of one molecule due to fluctuations in charge distribution in a second molecule and vice versa.

With the exception of highly polar substances, London dispersion forces account for nearly all of the Van der Waals attraction force. The London attraction between two molecules is short range and varies inversely with the sixth power of the intermolecular distance. For a collection of molecules the dispersion forces are approximately additive and the attractive energy between two particles can be computed by summing the attraction between all interparticle molecule pairs.

The results of such summations predict that the London attractive energy between an assembly of molecules (e.g. between colloidal particles) decays much less rapidly than between individual

molecules (Hamaker, 1937).

In the case of two identical spheres of radius, a, (in vacuo) with small separation, x (and when $x \ll a$), the energy of attraction V_A is given by:

$$V_A = \frac{-Aa}{12x} \qquad \text{---------} \quad (2)$$

where A is the Hamaker constant.

When the interparticle medium is not a vacuum A has to be replaced by an effective value of Hamaker constant, A^1, given by:

$$A^1 = (\sqrt{A_2} - \sqrt{A_1})^2 \qquad \text{---------} \quad (3)$$

Where subscripts 1 and 2 refer to dispersion medium and particles respectively.

The interparticle attraction will be weakest when the particles and the dispersion medium are chemically similar, since A_1 and A_2 will be of similar value and therefore A^1 will be low. More elaborate expressions have been reported by Schenkel & Kitchener (1960).

Qualitatively, the double layer interaction forces become important when particle separation is of the order of the double layer parameter, $^1/k$. The dispersion interactions are, for emulsion-sized particles, generally, negligible when the particle separation is of the order of the particle radius or more. It is difficult to

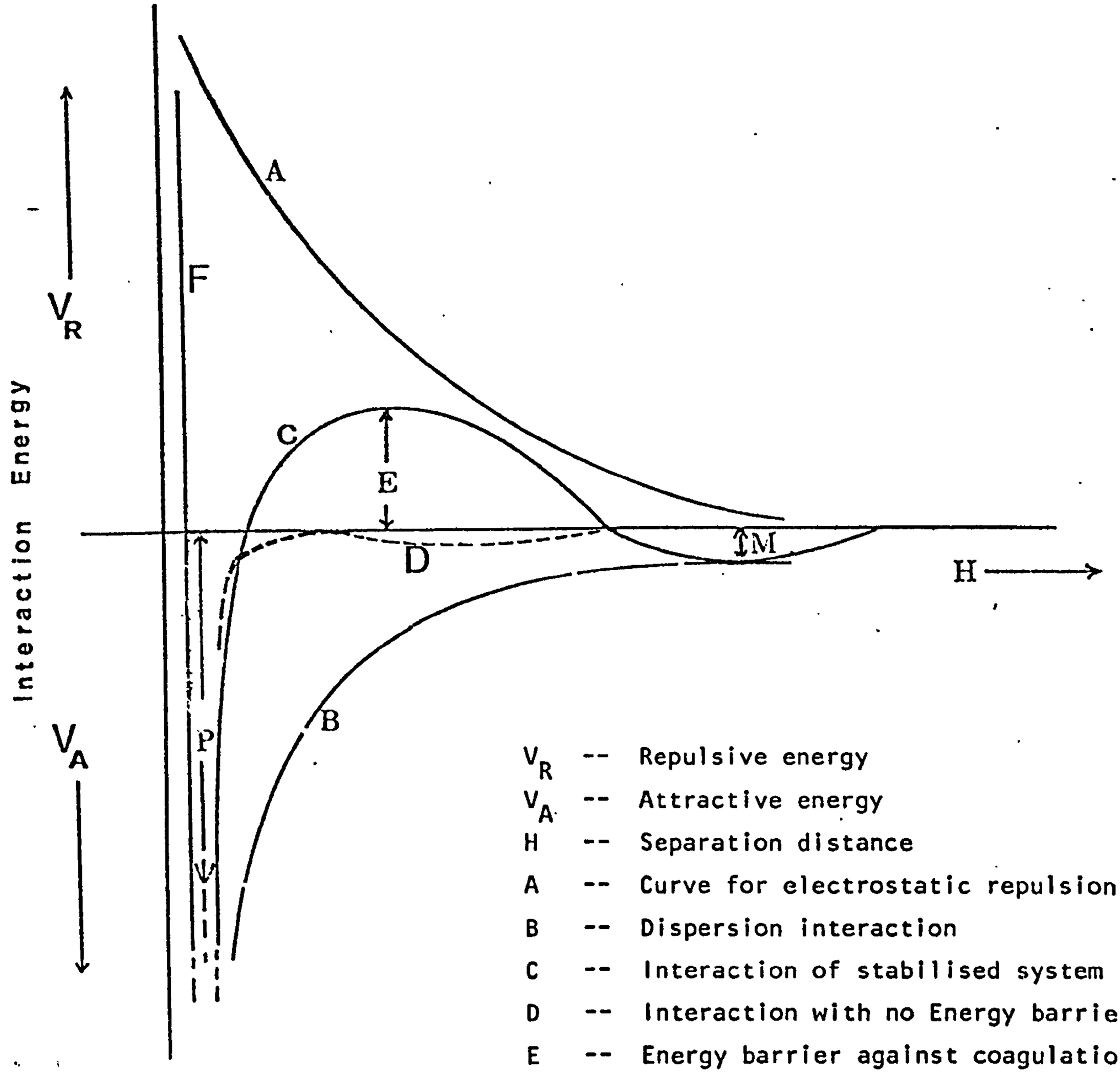
get absolute value for the total V_R V_A interaction, due to many variables present, e.g. solvation effects. It has been treated graphically and at length by Verwey & Overbeek (1948). The basic features are shown in Fig.35 , which depicts the quantities V_R (curve A), V_A (curve B) and two possible forms of $V = V_R + V_A$ (curves C & D).

Curves of type C show a nett repulsion between particles over a range of particle separation and therefore lead to a retardation of aggregation as compared with aggregation due to Brownian motion. The retardaton partly depends on the height, E, of the potential energy maximum. Curves of this type are typical of systems having a high surface potential and in which the electrolyte concentration is low.

Addition of electrolyte reduces the energy barrier E until the point is reached where no barrier remains. This point is reached before E = 0. When E = 0 (curve, D) aggregation is more rapid than Brownian aggregation owing to nett attractive forces. Aggregation of this type is generally in the primary minimum, P. It is emphasized here, that the problem is of diffusion of particles in a force field rather than of passage of separate particles over a potential energy barrier. Therefore it is not possible to say that a certain value of E would be sufficient for stability.

The treatment of the problem as one of diffusion is possible using Fuchs' method (1934), giving the expression:

Figure 35 Potential Energy of interaction between two particles as a function of separation.



V_R -- Repulsive energy
V_A -- Attractive energy
H -- Separation distance
A -- Curve for electrostatic repulsion
B -- Dispersion interaction
C -- Interaction of stabilised system
D -- Interaction with no Energy barrier
E -- Energy barrier against coagulation
F -- Born type interaction
P -- Primary minimum
M -- Secondary minimum

$$W = 2\int_{o}^{\infty} \frac{e^{-v/RT}}{H^2}\, dH \qquad \text{---------- (4)}$$

Where W = the stability ratio.

H = particle separation

T = absolute temperature

The value of W depends mainly on the value of V in the region of the potential energy maximum. This depends on the value of E. Values of E sufficient to ensure stability of even concentrated emulsions, of the order of 20 kT, have been suggested (Verwey & Overbeek, 1948).

Curve, C, in figure 3 5 shows a shallow minimum, M, (also called secondary minimum) at relatively large particle separation. Generally, this minimum is significant when particle sizes of about 1 μm or larger are involved, when M can be a few kiloteslas (Carroll, 1976). This minimum, therefore, features in many emulsified systems. In these systems flocculation is possible when particles are at the appropriate separation. The flocculation in the secondary minimum is characterized by its easy reversibility, e.g. on shaking. Flocculation complicates the kinetics of emulsion breakdown. Two flocculated droplets, in theory, should be more likely to coalesce than two isolated droplets in the same system. Therefore, an apparent increase in the coagulation rate kinetics is expected, which tends to mask the true coagulation rate into the primary minimum, (Cooper, 1972).

Modifications to the DLVO Theory.

Several of the proposed changes to the original DLVO theory are

system specific and therefore need not be applied simultaneously. These modifications are briefly discussed below.

1. Corrections to V_A

Relativistic retardation of the dispersion interaction reduces the attractive force as the particle separation increases. Therefore the expression for V_A derived by Hamaker needs to be corrected. The effect may be envisaged as the time taken for the fluctuation field from one atom to reach the second atom and the lifetime of the fluctuation. When these two times become of similar value the interaction decreases. Approximate expressions, for low and high particle separations have been reported by Schenkel & Kitchener (1960). V_A is modified depending upon the value of a parameter $P = 2\pi x/\lambda$, where λ is the London wavelength, as follows:

For the range $0 < P < 3$

$$V_A = \frac{Aa}{12x}\left(\frac{1}{1 + 1.77P}\right) \quad \text{---------} \quad (5)$$

For the range $3 < P < \infty$

$$V_A = \frac{Aa}{12x}\left(\frac{-2.45}{5P} + \frac{2.17}{15P^2} - \frac{0.59}{35P^3}\right) \quad \text{---------} \quad (6)$$

The correction is expected to be important when the particle separation exceeds the London Wavelength of electrons (~ 100 nm).

The effect of the adsorbed layer thickness at the interface on the dispersion interaction has been reported by Vold (1961).

She showed that appreciable reductions in the attractive force were sometimes to be expected and that the effect depends on the relative magnitudes of the Hamaker constants of the several components, the thickness of the adsorbed layer and on the particle radius.

Vold's treatment has been corrected by Vincent et al (1973), who considered the changes in V_A (due to adsorption of a layer of matter) under two headings: a Hamaker constant effect, arising from the difference in the values of the constant for the several different types of matter present; and a core effect, arising from the change in the spatial distribution of matter. The second effect is apparently more important. Vold was concerned primarily with the first effect. The core effect always brings about a reduction in the Van der Waal's attraction. The first effect is limited to cases of small particle size or small adsorbed layer thickness.

2. Modifications to V_R

The equation (2) for V_R is valid only when:

(a) the particle diameter is large compared with the double layer thickness, that is, when $Ka \gg 1$.

(b) when $KH \ll 1$.

The first condition is usually fulfilled in emulsion systems, but the second limits the use of the equation due to heterogeneity in particle type and size. A further important point is that the derivation of equation (2) assumes that

particles approach at constant surface charge rather than constant surface potential. Which of the two represents reality is still an open question; it may be system dependent.

3. Polydisperse systems

Early work ignored the effect of particle size heterogeneity on the DLVO-type particle interactons. Recently Cooper (1972) postulated an initially Gaussian distribution of particle size and investigated the variation in the overall stability ratio, W, with the standard deviation of the particle size distribution. From this study the following conclusions emerged:

(1) stability decreases with increased size heterogeneity.

(2) increase in the electrical surface potential tends to increase stability.

(3) variation in surface potential among particles decreases stability.

4. Viscous Interactions

The viscosity of the continuous phase enters the DLVO theory via the Einstein equation for the diffusion coefficient $D = kT/6\pi\eta a$ and the value of η (the viscosity coefficient) is usually assumed to be the bulk value. The use of D may not be justified for closely packed particles because:

(a) the fluid flow pattern around a closely packed particle is not the same as that around an isolated sphere in a continuum.

(b) the viscosity of the medium at small particle

separations may differ from the bulk viscosity.

For a more detailed account the reader is referred to the article by Spielman (1970).

5. Entropic and Related Forces

When particles approach very closely, interactions between the surfactant molecules at the surface of the particles is possible, especially if the surfactant molecules significantly protrude into the continuous phase. This happens in the case with W/O emulsion between the long hydrocarbon "tail" ends of the stabilizer and in the case of O/W emulsions when the stabilizers are certain nonionic surfactants (e.g. polyethoxylated ethers, $C_\eta E_x$) or macromolecules.

The details of what happens when interpenetration occurs is complex and is still a matter of discussion.

APPENDIX 2

The Gibbs Adsorption Equation

The Gibbs adsorption equation enables the estimation of the extent of adsorption of a substance at a liquid interface from the interfacial tension data.

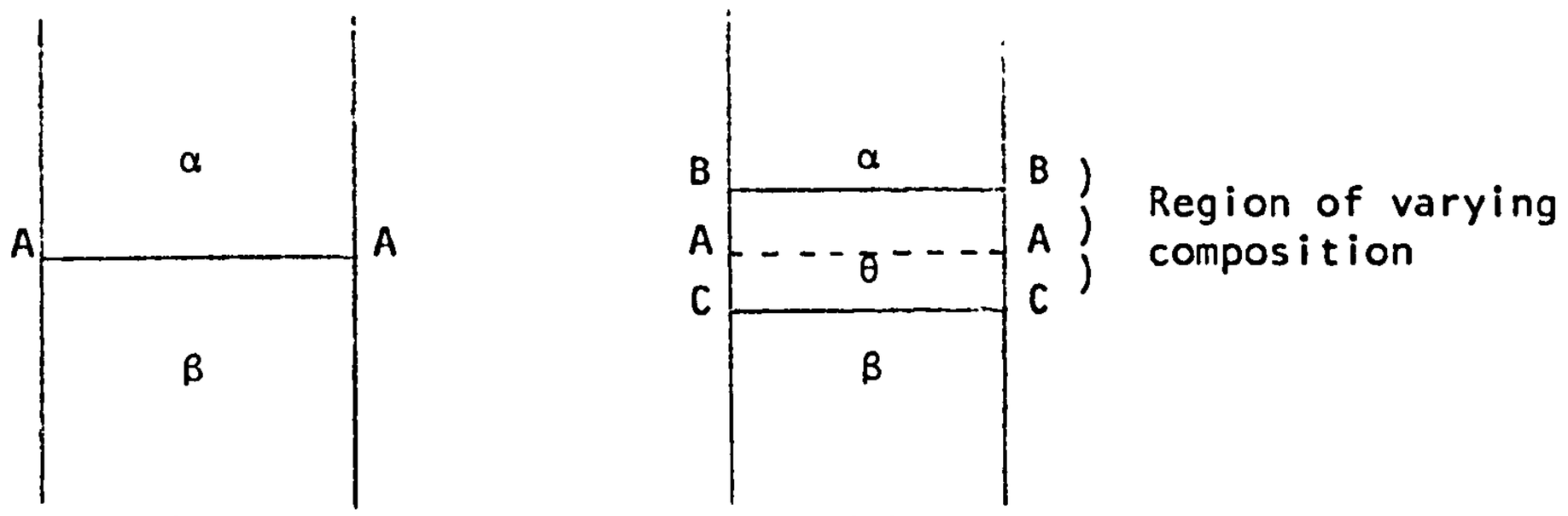


Diagram showing representations of an interface between bulk phases α and β.

In the derivation of the Gibbs adsorption equation it is generally assumed that the two phases, α and β, are divided by the interface A-A (see diagram above) having negligible thickness. But in reality, especially when an adsorbed film is present, there is an interfacial region of varying compositon having a certain thickness of molecular dimensions.

The plane A-A is also sometimes called the Gibbs surface and at this surface adsorption may be described in terms of the surface excess concentration (Γ). If n_i^θ is the amount of component, i, in the surface phase, θ, (see diagram above) in excess of the concentration which would have been present if the bulk phases had extended to the plane A-A with unchanged composition, the surface excess of the component, i, is given by:

$$\Gamma_i = \frac{n_i^\theta}{A} \quad \text{---------- (1)}$$

where A is the interfacial area.

From free energy considerations, the expression for the thermodynamic energy of the surface phase, θ, can be written as:

$$U^\theta = TS^\theta - PV^\theta + \gamma A + \Sigma \mu_i \, n_i^\theta \quad \text{--------- (2)}$$

where T is the absolute temperature

S^θ is the entropy of phase θ

γ is the surface tension

μ_i the chemical potential of i.

The terms PV^θ and γA have opposite signs, since pressure is an expanding force and surface tension a contracting force.

Differentiating equation (2) we get

$$dU^\theta = TdS^\theta + S^\theta dT - PdV^\theta - V^\theta dP + \gamma dA + Ad\gamma + \Sigma \, \mu_i dn_i^\theta$$

$$+ \Sigma \, n_i^\theta d\mu_i \quad \text{--------- (3)}$$

From the first and second laws of thermodynamics:

$$dU^\theta = TdS^\theta - PdV^\theta + \gamma dA + \Sigma \mu_i dn_i^\theta \quad \text{--------- (4)}$$

Subtracting equation (4) from (3) we get:

$$S^\theta dT - V^\theta dP + Ad\gamma + \Sigma \, n_i^\theta d\mu_i = 0 \quad \text{--------- (5)}$$

Therefore at constant temperature and pressure:

$$d\gamma = - \Sigma \frac{n_i^\theta}{A} d\mu_i = - \Sigma \Gamma_i d\mu_i \quad \text{--------- (6)}$$

For 2 component solution equation (6) becomes:

$$d\gamma = - \Gamma_A \, d \, \mu_A - \Gamma_B \, d \, \mu_B \quad \text{--------- (7)}$$

where subscripts A and B refer to the two components.

The surface excess concentrations are defined relative to an arbitrarily chosen dividing surface (A - A), and generally the location (seemingly realistic) of this surface for a binary solution is chosen where the surface excess concentration of the solvent (Γ_A) is zero, in this case equation (7) becomes:

$$d\gamma = - \Gamma_B \, d \, \mu_B \quad \text{--------- (8)}$$

Since chemical potential changes are related to relative activities by:

$$\mu_B = \mu_B^{\theta} + RT \ln a_B \quad \text{--------- (9)}$$

then

$$d\mu_B = RT \, d \ln a_B \quad \text{--------- (10)}$$

where a_B is the activity of component B.

Therefore

$$\Gamma_B = - \frac{1}{RT} \cdot \frac{d\gamma}{d \ln a_B} = - \frac{a_B}{RT} \cdot \frac{d\gamma}{da_B} \quad \text{--------- (11)}$$

For dilute solutions the activity may be replaced by concentration so that:

$$\Gamma_B = - \frac{C_B}{RT} \cdot \frac{d\gamma}{dC_B} \quad \text{--------- (12)}$$

Equation 12 is how the Gibbs equation is usually quoted.

APPENDIX 3

TABLE II

Table of $1/H$ in Terms of S; S. Fordham, *Proc. Roy. Soc.* (*London*), 194A, 1 (1948)

S	0	1	2	3	4	5	6	7	8	9
0.66	0.93828	0.93454	0.93082	0.92712	0.92345	0.91979	0.91616	0.91255	0.90895	0.90538
0.67	0.90183	0.89830	0.89478	0.89129	0.88782	0.88436	0.88092	0.87751	0.87411	0.87073
0.68	0.86737	0.86403	0.86070	0.85739	0.85410	0.85083	0.84758	0.84434	0.84112	0.83792
0.69	0.83473	0.83156	0.82841	0.82527	0.82215	0.81905	0.81596	0.81289	0.80983	0.80679
0.70	0.80376	0.80075	0.79776	0.79478	0.79182	0.78887	0.78594	0.78302	0.78011	0.77722
0.71	0.77435	0.77149	0.76864	0.76581	0.76300	0.76019	0.75741	0.75463	0.75187	0.74912
0.72	0.74639	0.74367	0.74097	0.73828	0.73560	0.73293	0.73028	0.72764	0.72502	0.72240
0.73	0.71980	0.71722	0.71464	0.71208	0.70953	0.70700	0.70447	0.70196	0.69946	0.69697
0.74	0.69449	0.69202	0.68957	0.68713	0.68470	0.68228	0.67988	0.67748	0.67510	0.67273
0.75	0.67037	0.66803	0.66569	0.66337	0.66105	0.65875	0.65646	0.65418	0.65191	0.64965
0.76	0.64740	0.64516	0.64294	0.64072	0.63851	0.63632	0.63413	0.63195	0.62979	0.62763
0.77	0.62549	0.62335	0.62122	0.61911	0.61700	0.61490	0.61281	0.61074	0.60867	0.60661
0.78	0.60457	0.60253	0.60050	0.59848	0.59647	0.59447	0.59248	0.59049	0.58852	0.58656
0.79	0.58460	0.58265	0.58072	0.57879	0.57687	0.57496	0.57305	0.57116	0.56927	0.56739
0.80	0.56553	0.56366	0.56181	0.55997	0.55813	0.55630	0.55448	0.55266	0.55086	0.54906
0.81	0.54727	0.54549	0.54371	0.54195	0.54019	0.53844	0.53669	0.53496	0.53323	0.53151
0.82	0.52979	0.52808	0.52638	0.52469	0.52300	0.52132	0.51965	0.51799	0.51634	0.51469
0.83	0.51305	0.51142	0.50979	0.50817	0.50656	0.50496	0.50336	0.50176	0.50018	0.49860
0.84	0.49703	0.49546	0.49390	0.49234	0.49080	0.48926	0.48772	0.48619	0.48467	0.48316
0.85	0.48165	0.48015	0.47865	0.47716	0.47567	0.47420	0.47272	0.47126	0.46980	0.46834
0.86	0.46690	0.46545	0.46402	0.46259	0.46116	0.45974	0.45833	0.45692	0.45552	0.45412
0.87	0.45273	0.45134	0.44996	0.44858	0.44721	0.44584	0.44448	0.44313	0.44178	0.44044
0.88	0.43910	0.43777	0.43644	0.43512	0.43380	0.43249	0.43118	0.42988	0.42858	0.42729
0.89	0.42600	0.42471	0.42344	0.42216	0.42089	0.41963	0.41837	0.41712	0.41587	0.41462
0.90	0.41338	0.41214	0.41091	0.40968	0.40846	0.40724	0.40602	0.40481	0.40360	0.40240
0.91	0.40121	0.40001	0.39882	0.39764	0.39646	0.39528	0.39411	0.39294	0.39177	0.39061
0.92	0.38946	0.38831	0.38716	0.38601	0.38487	0.38374	0.38260	0.38147	0.38035	0.37923
0.93	0.37810	0.37699	0.37588	0.37477	0.37366	0.37256	0.37146	0.37037	0.36928	0.36819
0.94	0.36711	0.36602	0.36494	0.36387	0.36280	0.36173	0.36066	0.35960	0.35854	0.35748
0.95	0.35643	0.35538	0.35433	0.35328	0.35224	0.35120	0.35016	0.34913	0.34809	0.34706
0.96	0.34604	0.34501	0.34399	0.34297	0.34195	0.34093	0.33992	0.33890	0.33789	0.33688
0.97	0.33588	0.33487	0.33387	0.33287	0.33186	0.33086	0.32987	0.32887	0.32787	0.32688
0.98	0.32588	0.32489	0.32389	0.32290	0.32191	0.32092	0.31992	0.31893	0.31794	0.31695
0.99	0.31595	0.31496	0.31396	0.31296	0.31196	0.31095	0.30994	0.30893	0.30792	0.30690
1.00	0.30588	0.30484	0.30381	0.30276	—	—	—	—	—	—

TABLE III

Table of $1/H$ in Terms of S; C. E. Stauffer, *J. Phys. Chem.*, 69, 1933 (1965)

S	0	1	2	3	4	5	6	7	8	9
0.30	7.09837	7.03966	6.98161	6.92421	6.86746	6.81135	6.75586	6.70099	6.64672	6.59306
0.31	6.53998	6.48748	6.43556	6.38421	6.33341	6.28317	6.23347	6.18431	6.13567	6.08756
0.32	6.03997	5.99288	5.94629	5.90019	5.85459	5.80946	5.76481	5.72063	5.67690	5.63364
0.33	5.59082	5.54845	5.50651	5.46501	5.42393	5.38327	5.34303	5.30320	5.26377	5.22474
0.34	5.18611	5.14786	5.11000	5.07252	5.03542	4.99868	4.96231	4.92629	4.89061	4.85527
0.35	4.82029	4.78564	4.75134	4.71737	4.68374	4.65043	4.61745	4.58479	4.55245	4.52042
0.36	4.48870	4.45729	4.42617	4.39536	4.36484	4.33461	4.30467	4.27501	4.24564	4.21654
0.37	4.18771	4.15916	4.13087	4.10285	4.07509	4.04759	4.02034	3.99334	3.96660	3.94010
0.38	3.91384	3.88786	3.86212	3.83661	3.81133	3.78627	3.76143	3.73682	3.71242	3.68824
0.39	3.66427	3.64051	3.61696	3.59362	3.57047	3.54752	3.52478	3.50223	3.47987	3.45770
0.40	3.43572	3.41393	3.39232	3.37089	3.34965	3.32858	3.30769	3.28698	3.26643	3.24608
0.41	3.22582	3.20576	3.18587	3.16614	3.14657	3.12717	3.10794	3.08886	3.06994	3.05118
0.42	3.03258	3.01413	2.99583	2.97769	2.95969	2.94184	2.92415	2.90659	2.88918	2.87192
0.43	2.85479	2.83781	2.82097	2.80426	2.78769	2.77125	2.75496	2.73880	2.72277	2.70687
0.44	2.69110	2.67545	2.65992	2.64452	2.62924	2.61408	2.59904	2.58412	2.56932	2.55463
0.45	2.54005	2.52559	2.51124	2.49700	2.48287	2.46885	2.45494	2.44114	2.42743	2.41384
0.46	2.40034	2.38695	2.37366	2.36047	2.34738	2.33439	2.32150	2.30870	2.29600	2.28339
0.47	2.27088	2.25846	2.24613	2.23390	2.22176	2.20970	2.19773	2.18586	2.17407	2.16236
0.48	2.15074	2.13921	2.12276	2.11640	2.10511	2.09391	2.08279	2.07175	2.06079	2.04991
0.49	2.03910	2.02838	2.01773	2.00715	1.99666	1.98623	1.97588	1.96561	1.95540	1.94527
0.50	1.93521	1.92522	1.91530	1.90545	1.89567	1.88596	1.87632	1.86674	1.85723	1.84778
0.51	1.83840	1.82909	1.81984	1.81065	1.80153	1.79247	1.78347	1.77453	1.76565	1.75683
0.52	1.74808	1.73938	1.73074	1.72216	1.71364	1.70517	1.69676	1.68841	1.68012	1.67188
0.53	1.66369	1.65556	1.64748	1.63946	1.63149	1.62357	1.61571	1.60790	1.60014	1.59243
0.54	1.58477	1.57716	1.56960	1.56209	1.55462	1.54721	1.53985	1.53253	1.52526	1.51804
0.55	1.51086	1.50373	1.49665	1.48961	1.48262	1.47567	1.46876	1.46190	1.45509	1.44833
0.56	1.44158	1.43489	1.42825	1.42164	1.41508	1.40856	1.40208	1.39564	1.38924	1.38288
0.57	1.37656	1.37028	1.36404	1.35784	1.35168	1.34555	1.33946	1.33341	1.32740	1.32143
0.58	1.31549	1.30958	1.30372	1.29788	1.29209	1.28633	1.28060	1.27491	1.26926	1.26365
0.59	1.25805	1.25250	1.24698	1.24149	1.23603	1.23061	1.22522	1.21987	1.21454	1.20925
0.60	1.20399	1.19875	1.19356	1.18839	1.18325	1.17814	1.17306	1.16801	1.16300	1.15801
0.61	1.15305	1.14812	1.14322	1.13834	1.13350	1.12868	1.12389	1.11913	1.11440	1.10969
0.62	1.10501	1.10036	1.09574	1.09114	1.08656	1.08202	1.07750	1.07300	1.06853	1.06409
0.63	1.05967	1.05528	1.05091	1.04657	1.04225	1.03796	1.03368	1.02944	1.02522	1.02103
0.64	1.01684	1.01269	1.00856	1.00446	1.00037	0.99631	0.99227	0.98826	0.98427	0.98031
0.65	0.97635	0.97242	0.96851	0.96463	0.96077	0.95692	0.95310	0.94930	0.94553	0.94177
0.66	0.93803	0.93431	0.93061	0.92693	0.92327	0.91964	0.91602	0.91242	0.90884	0.90528

APPENDIX 4

Theory of Molecular Diffusion

The theory of molecular diffusion is based on the fact that the vapour pressure of a liquid droplet is higher than the saturated vapour pressure over the plane surface of that liquid. In terms of fugacity (f) we may write:

$$RT \ln (f_d/f_\infty) = 4 \gamma V/d \quad \text{--------- (1)}$$

where, f_d, is the fugacity of a droplet of diameter, d, and, f_∞, the fugacity of the flat surface. γ is the surface tension and V the molar volume.

For the single droplets of a sparingly water soluble oil, obeying Henry's law, such as a fluorocarbon oil, equation (1) can be expressed as:

$$RT \ln (C_d/C_\infty) = 2 \gamma_{ow} M/r\rho \quad \text{--------- (2)}$$

Where C_d and C_∞ are the respective solubilities of the droplet of radius, r and infinity, γ_{ow} is the interfacial tension, M and ρ are the molecular weight and density respectively of the oil. Provided that γ_{ow} and ρ are constant for small particles, equation (2) simplifies to:

$$C_d = C_\infty \text{ exp. } (K_a/r) \quad \text{--------- (3)}$$

There is evidence that although K_a is approximately constant, γ_{ow} and ρ vary with particle size, (Davis & Smith 1976).

Higuchi and Misra (1962) have reported an analysis of the dissolution of small droplets and growth of the larger ones. The dissolution rate for spherical particles controlled by diffusion can be expressed as:

$$G = 4\pi\ D\ r\ (C_s - C_o) \quad \text{--------- (4)}$$

where

G is the rate of dissolution

D = diffusion coefficient of droplet material in the continuous phase.

C_s = solubility of the growing or dissolving phase.

C_o = concentration of the droplet material in the external phase at a point remote from the droplet.

Substituting equation (3) into equation (4):

$$G = 4\pi\ D\ r\ [C_\infty \text{ exp. } (K_a/r) - C_o] \quad \text{--------- (5)}$$

Using equation (5) and taking into account the mass of the particle material dissolved in the system, the rate of change of a droplet can be estimated (Higuchi & Misra, 1962).

For a system, containing particles of two radii, r_1 and r_2, which grow at rate G_1 and dissolve at rate G_2 respectively, the rate of decrease of r_2 will be

$$dr_2/dt = \frac{D\ C_\infty\ K_a}{r_2^2}\left[\frac{n_2\ (r_2 - r_1)}{n_1 r_1 - n_2 r_2}\right] \quad \text{--------- (6)}$$

Where n_1 and n_2 are the numbers of particles of radius r_1 and r_2 respectively. Based on their calculations Higuchi and

Misra (1962) have concluded that molecular diffusion may be important even for sparingly water soluble oils in systems having a particle size of 1 μm or less. The presence of dissolved external phase has been shown to have negligible effect on the molecular diffusion rate by these workers.

They also considered the effect of a third non-interacting component (y) dissolved in the disperse phase (x) and concluded that the component, y, will give rise to state of equilibrium where the rate of degradation depends on the diffusion rate of x or y or both. If the diffusion coefficients, D_x and D_y are similar and $K_d(x) \gg K_d(y)$ (where K_d is partition coefficient and x and y refer to the oil phase and the component, y, respectively) the degradation rate will be similar to that of x alone.

But if $K_d(x) \ll K_d(y)$ then the diffusion of the component y will control the process. It was suggested that degradation rate will be retarded by a factor F_d given by:-

$$F_d = K_d(y)/K_d(x) \quad \text{---------(7)}$$

Thus, the higher the F_d, the more stable the system will be to molecular diffusion.

APPENDIX 5

Glossary of Symbols and Abbreviations

d_{vs}	mean (volume - surface) droplet diameter
Pf	Perfluoro -
ϵ, a	Surfactant concentration and activity respectively.
D	droplet diameter
d_{av}	mean (length - number) droplet diameter of emulsion
D_t/D_o	Ratio of mean droplet diameter at a particular time, t, to the initial mean diameter of emulsion droplets
I.U.P.A.C.	International Union of Physicists and Chemists
F_d	Emulsion stability factor
g	Acceleration due to gravity (9.81 m s^{-2})
H	distance of separation between two interacting particles
h	distance of separation between droplet and plane interface
k	Boltzmann constant
N	number of droplets not coalesced at a plane interface after a given time
N_o	Number of droplet rest-times measured
n_i	Excess number of molecules of i th. component in an interface
P	gas pressure
ρ	density
ρ_o	density of dispersed oil
R	gas constant (8.314 J K^{-1} mol^{-1})
r	droplet radius

S	specific interface of emulsion
s	spreading coefficient
T	Absolute temperature
$T_{\frac{1}{2}}$	First order half-life from droplet rest-time data
t	time (droplet rest-time or emulsion age)
t_d	drainage time for film between single droplet and plane interface
t_h	time required for film to drain to thickness h
t_1, t_2	respective flow times of Phases 1 and 2 through U-tube viscometer
V	volume
V_A	Attractive interaction between two particles
V_R	repulsive interaction between two particles
V_T	Total interaction between two particles
W_A	Work of adhesion
W_C	Work of cohesion
CmC	Critical micelle concentration
DLVO	Derjaguin-Landau and Verwey-Overbeek theory
GLC	gas-liquid chromatography
HLB	hydrophilic-lipophilic balance
O-W	oil-water interface
O/W	oil in water (emulsion)
PIT	Phase inversion temperature
rpm	revolutions per minute
SDDS	sodium dodecyl sulphate
W/O	water-in-oil (emulsion)
W/O/W	water-in-oil-in-water (multiple emulsion)
Γ	Surface excess of solute

γ	Surface or interfacial tension
γ_o, γ_w	respective surface tensions of oil and water
γ_{ow}	Interfacial tension between oil and water phases
$\gamma_{1,2}$	Interfacial tension between phases 1 and 2
$\Delta\rho$	density difference between two phases
ϵ	dielectric constant
ω	centrifugal force
η	viscosity
η_r	relative viscosity
μ_i	surface chemical potential of adsorbed species
π	*surface or interfacial* pressure (Film pressure)
ψ_o	surface potential
P_o	Vapour pressure of plane surface of liquid
P_r	Vapour pressure of droplet of radius, r
V_m	the molar volume
N_A	Avogadro number
A	area per adsorbed surfactant molecule
FC	Fluorocarbon
LD_{so}	Lethal dose to 50% of the group of animals
l	length (of Wilhelmy plate)
l_o	End-Correction (Wilhelmy plate)
d_e	Equatorial pendant drop diameter
d_s	pendant drop diameter at a distance of d_e from the bottom of the drop
S	ratio d_s/d_e used to calculate interfacial tension
γ_i	interfacial tension
k_c	Coalescence rate constant
α	Coalescence constant (single droplet)
σ	Standard deviation

E	Electrode potential
z	Valency of ion
F	Faraday number
A_{F^-}	activity of fluoride ions
F^-	The fluoride ion
pF	is -log A_{F^-}
v	sedimentation velocity
ρ_m, ρ_e	densities of the medium and emulsion particle respectively
x	distance (equation 4, page 114)
S.G.	Specific Gravity
γ_w^d	The dispersion interactions contribution to the surface tension of water
e	Electronic charge
A^1	Hamaker constant

LIST OF FIGURES